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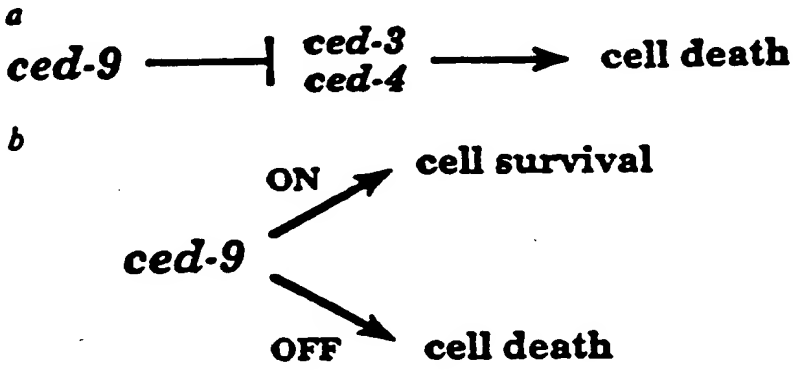

(57) Abstract

The present invention relates to genes, referred to herein as cell death-protective genes, which protect cells against programmed cell death by antagonizing the activities of genes which cause cell death. As described herein, a cell death-protective gene from the nematode *Caenorhabditis elegans*, called *ced-9*, has been identified, sequenced, and characterized. *ced-9* is essential for *C. elegans* development and apparently functions by protecting cells which normally live during development from programmed cell death. Mutations which constitutively activate and inactivate the *ced-9* gene are also described. *ced-9* was shown to function by antagonizing the activities of the cell death genes, *ced-3* and *ced-4*. As further described, the protein product of the human oncogene *bcl-2* was found to have a similar sequence to the *ced-9* protein. Methods and agents for both increasing and decreasing the occurrence of cell death are described that are potentially useful for diagnosis, prevention and therapy of diseases and conditions involving cell death; for the treatment of viral, parasitic, and other types of infection; and for killing organisms that are detrimental or potentially detrimental to the environment or to humans, pets, livestock, or agriculture.

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A GENE WHICH PREVENTS PROGRAMMED CELL DEATH

Background

Cell death is a fundamental aspect of animal development. A considerable proportion of the cells that are generated die during the normal development of both vertebrates (Glucksmann, Biol. Rev. Cambridge Philos. Soc. 26:59-86 (1951)) and invertebrates (Truman and Schwartz, Ann. Rev. Neurosci. 7:171-188 (1984)). Cell death plays a role in morphogenesis (e.g., of the eye, secondary palate, heart, nervous system and limbs in vertebrate embryos), metamorphosis (e.g., in moths and other insects), and tissue homeostasis (e.g., of epithelial linings and the thymus), as well as in neuron selection during the establishment of synaptic connections and in sexual dimorphism (reviewed by Ellis et al., Ann. Rev. Cell Biol. 7:663-698 (1991)). Cell death which occurs as a part of normal development will be referred to herein as physiological cell death.

Besides physiological cell death, cell death may occur as a pathological manifestation of disease, in which case it will be referred to herein as pathological cell death (see review by Trump and Mergner (1974), in: The Inflammatory Process, vol. 1, 2nd ed. (eds. Zweifach et al.), Academic Press, New York, pp. 115-257). Cell death can result from a variety of injuries to the cell, including toxins, ischemia (lack of blood supply), hypoxia (lack of oxygen) and infectious agents, as well as from genetic mutations. The major clinical aspects of most degenerative diseases are a consequence of cell death. For example, Huntingtons's disease, Parkinson's disease, Alzheimer's disease and amyotrophic lateral sclerosis are marked by degeneration of neurons, while Duchenne muscular dystrophy is characterized by muscle degeneration. In addition, some cancers are thought to be caused by a defect in cell death processes. Thus,

understanding and preventing cell death can be viewed as one of the major goals of biomedical research.

The simple and invariant anatomy and development of the nematode Caenorhabditis elegans have made it an attractive system for the study of cell death. Because C. elegans is small, cellularly simple and transparent, Nomarski differential interference microscopy can be used to observe individual cells throughout development. As a result, the complete cell lineage of C. elegans, from zygote to adult, has been elucidated (Sulston and Horvitz, Dev. Biol. 82:110-156 (1977); Kimble and Hirsh, Dev. Biol. 70:396-417 (1979); Sulston et al., Dev. Biol. 100:64-119 (1983)).

Cell death is an important component of the development of C. elegans: during the development of the adult hermaphrodite, the generation of 816 nongonadal cells is accompanied by the generation and subsequent deaths of an additional 131 cells. Cell death appears to be an integral part of the differentiation of a variety of tissues. The pattern of cell deaths is essentially invariant among different animals, i.e, the same set of cells die at the same developmental time. In addition, a vast majority of cell deaths in C. elegans does not appear to be initiated by interaction with surrounding cells or diffusible factors.

Genetic analysis has identified many genes that affect programmed cell death in C. elegans (reviewed by Ellis et al. (1991) supra). The activities of two genes, ced-3 and ced-4, seem to be required for the onset of almost all C. elegans programmed cell deaths (Ellis and Horvitz, Cell 44:817-829 (1986)). Mutations in ced-3 and ced-4 block essentially all programmed cell deaths. In ced-3 and ced-4 mutants, cells that normally undergo programmed cell death instead survive, differentiate and even function (Ellis and Horvitz

(1986) supra; Avery and Horvitz, Cell 51:1071-1078 (1987); White et al., Phil. Trans. R. Soc. Lond. B. 331:263-172 (1991)). Genetic analyses indicate that ced-3 and ced-4 genes most likely act within dying cells; this suggests that of these genes are expressed within dying cells and either encode cytotoxic molecules or control the activities of cytotoxic molecules (Yuan and Horvitz, Dev. Biol. 138:33-41 (1990)).

Relatively little is known about the mechanism of cell death. Initiation of cell death occurs in response to a variety of signals. External injuries and cytotoxic agents cause cells to die. Endocrine signals trigger cell death during insect metamorphosis, thymocyte death and regression of the prostate in the male rat after castration. Lack of neuronal growth factors is suspected to be the cause of certain neuronal cell deaths during vertebrate development and may also be the cause of cell deaths in certain neurodegenerative diseases. A specific protein, Mullerian inhibiting substance, is responsible for the regression of the Mullerian duct during the development of male mammals. In addition, genetically programmed cell deaths which occur apparently autonomously of cell-cell interaction or diffusible factors are observed in C. elegans and other invertebrates. (Truman and Schwartz, Neuro. Comm. 1:66-72 (1982); Cohen and Duke, J. Immunol. 132:38-42 (1984); Isaacs, Prostate 5:545-557 (1984); Martin et al., J. Cell. Biol. 106:829-844 (1988); Oppenheim and Prevette, Neurosci. Abstr. 14:368 (1988); Beal et al., Nature 321:168-171 (1986); Birkmayor and Hornykiewicz, Advances in Parkinsonism, Fifth International Symposium on Parkinson's Disease, Vienna, Roche, Basle, 1976; Lagsto et al., Science 219:979-980 (1983); Rossor, Lancet 2:1200-1204 (1982); Biel et al., Science 229:289-291 (1985); Cosi et al., in: Advances in Experimental

Medicine and Biology, vol. 209, Plenum Press, New York, 1987; Bonilla et al., Cell 54:447-452 (1988); Picard and Josso, Biomedicine 25:147-150 (1976)).

Cell deaths also vary morphologically. Two major categories of cell deaths have been established based on morphological features (Kerr et al., Br. J. Cancer 26:239-257 (1972)). The first type of cell death, called necrosis, is characterized by cellular swelling, rupture of plasma and internal membranes, and eventual leakage of cellular contents into the extracellular space. The second, called apoptosis, involves progressive condensation of cytoplasm and nuclear chromatin and eventual fragmentation of cellular membranes into 'apoptotic bodies', which are usually digested by macrophages or adjacent epithelial cells. Necrosis is most often a manifestation of certain pathological conditions, e.g., injury by complement (Hawkins et al., Am. J. Pathol. 68:255-288 (1972)), hypoxia (Jennings et al., Am. J. Pathol. 81:179-198 (1975)), or exposure to a variety of toxins (McLean et al., Int. Rev. Exp. Pathol. 4:127-157 (1965)). In contrast, apoptosis is usually associated with physiological conditions, e.g., embryogenesis (Bellaris, J. Anat. 95:54-60 (1961); Saunders, Science 154:604-612 (1966)) and metamorphosis (Truman, Ann. Rev. Neurosci. 7:171-188 (1984). Interestingly, morphological features of physiological cell death in C. elegans resemble, in general, those of apoptosis in vertebrates (Ellis et al., Ann. Rev. Cell Biol. 7:663-698 (1991)). However, deviations from the standard descriptions of necrosis and apoptosis are often observed. It is uncertain whether this morphological classification reflects real differences in underlying mechanisms of cell death.

Although the initiation and morphology of cell death vary, there is evidence which suggests that most

physiological and some pathological cell deaths may share a common feature involving the activation of cell death genes. The existence of a genetic cell death program in a variety of organisms is suggested by the observation that protein and RNA synthesis inhibitors can prevent or delay a variety of cell deaths (insect metamorphosis, prostate regression, vertebrate neuronal cell death and thymocyte cell death) (Lockshin, J. Insect Physiol. 15:1505-1516 (1969); Stanisic et al., Invest. Urol. 16:19-22 (1978); Martin et al. (1988) supra; Oppenheim and Prevette (1988) supra; Cohen and Duke (1984) supra). New RNA and protein species have been found after the initiation of cell death in the rat prostate after castration (Buttyan et al., Molecular Endocrinology 2:650-657 (1988); Lee et al., Prostate 7:171-185 (1985)). Thus, a better understanding of the mechanisms of cell death would have wide biological application and provide a basis for altering or controlling the process.

Summary of the Invention

The present invention relates to genes, referred to herein as cell death-protective genes, which function to protect cells against programmed cell death by antagonizing the activity of genes which cause cell death. As described herein, Applicants have identified what appears to be a key or master regulatory gene whose activity determines whether a cell survives or undergoes cell death.

Specifically, a cell death-protective gene from the nematode Caenorhabditis elegans, called ced-9, has been identified, sequenced, and characterized. ced-9 is essential for C. elegans development and apparently functions by protecting cells which normally live during development from programmed cell death. As is also

described herein, a mutation that constitutively activates ced-9 prevents cells which normally die during development from undergoing programmed cell death, and mutations that inactivate ced-9 result in the deaths of cells which normally survive during development and consequently, in embryo lethality. ced-9 has been shown to function by antagonizing the activities of the cell death genes ced-3 and ced-4. Thus, the C. elegans ced-9 gene appears to act as a binary switch to regulate programmed cell death. Results described herein indicate that many and possibly all cells that survive during C. elegans development do so because ced-9 activity prevents them from undergoing programmed cell death.

In addition, a human equivalent of the C. elegans ced-9 gene has been discovered. The deduced amino acid sequence of the ced-9 gene product was found to have about 23% identity and about 47% similarity to the product of the human oncogene bcl-2. This structural similarity, together with previous studies on bcl-2 activity in lymphocytes, strongly suggests that bcl-2 is a human equivalent of ced-9. Applicants further provide methods for identifying other cell death-protective genes from a variety of organisms, including vertebrates (e.g., mammals and particularly humans), invertebrates (e.g., insects), microbes (e.g., yeast), and possibly plants. Furthermore, comparison of ced-9, bcl-2, and other cell death-protective genes and their encoded products provides a way to define key functional features or regions of these genes and gene products. Those features or parts that are conserved between these genes or their gene products are most likely to be functionally important.

Applicants further provide methods and agents for altering the occurrence of cell death in a population of

cells and hence, affecting the proliferative capacity and longevity of tissues or organisms. Methods and agents for both decreasing and increasing cell deaths are described. The agents may be all or portions of the cell death-protective genes and encoded products, or derivatives, mimetics, activators or inactivators, or agonists or antagonists of the activity of cell death-protecting genes.

As a result of this work, methods and agents for altering cell death are available for therapeutic or preventive treatment of diseases or conditions involving cell death. Methods and agents for reducing cell death are available and are potentially useful for treating disorders and conditions, including those associated with aging, hair loss, stroke, traumatic brain injury, myocardial infarction, degenerative diseases (including Huntington's disease, amyotropic lateral sclerosis, Alzheimer's disease, Parkinson's disease, and Duchenne's muscular dystrophy) and viral and other types of infection (such as with the human immunodeficiency virus or HIV). Methods and agents for increasing cell deaths are also available which are potentially useful for decreasing the growth of or for killing specific cell populations, such as infected cells or autoreactive immune cells. These methods and agents may also be useful for treating diseases or conditions characterized by excessive cell growth or an abnormally low frequency of cell death (e.g., neoplasia and other cancerous growth). Methods and agents which increase cell death are also potentially useful for treating viral, parasitic, and other infections and to kill undesirable organisms, for example, in pest control or biological containment applications.

Brief Description of the Drawings

Figure 1 is a diagrammatic representation of a model for ced-9 function. (a) ced-9 is a negative regulator of ced-3 and ced-4 activity. (b) ced-9 acts as a binary switch to regulate programmed cell death. When ced-9 is active, the activities of ced-3 and ced-4 are blocked, and the cell survives. When ced-9 is inactive, ced-3 and ced-4 are activated, leading to programmed cell death.

Figures 2A-J show the nucleotide sequence (SEQ ID NO:1) of the genomic region containing the C. elegans ced-9 gene, with selected restriction sites.

Figure 3 shows the nucleotide sequence (SEQ ID NO:2) of a particular ced-9 cDNA, with selected restriction sites and the predicted translation product.

Figure 4 shows the predicted amino acid sequence (SEQ ID NO:3) of the Ced-9 protein as deduced from the genomic and cDNA sequences.

Figure 5 shows changes observed in several ced-9 mutants. Shown are changes in the DNA sequence and the resulting predicted change in the protein sequence associated with each mutation.

Figure 6 shows the optimized alignment of the C. elegans Ced-9 (SEQ ID NO:3) and human Bcl-2 (SEQ ID NO:5) proteins. Identical residues are indicated by vertical bars between the sequences, and similar residues are indicated by one or two dots (. or :), for weak and strong similarity, respectively. A residue that is mutated in the gain-of-function allele n1950 is conserved and has been boxed. Residues mutated in the loss-of-function alleles, n1653ts and n2077, are also indicated by boxes.

Figures 7A-E show the cDNA sequence of bcl-2 (SEQ ID NO:4). The coding sequence is from nucleotides 1459 to 2178, inclusive.

Figure 8 shows the screen for mutations that result in a loss of ced-9 function.

Figure 9 is a diagram of the ced-9 cloning strategy and cosmid rescue. a) Genetic map of the ced-9 region. Relevant genes as well as the approximate position of the N2/RC301 restriction fragment length polymorphisms (RFLPs) used to map ced-9 are shown. b) Number of recombination events observed between various markers in the unc-69 to unc-49 interval. The nP55 polymorphism did not separate from unc-69 in these experiments, suggesting that unc-69 is to the right, or close and to the left of nP55.
c) Cosmid rescue of unc-69 and ced-9. Cosmids situated between the nP55 and nP56 RFLPs (recognized by cosmids C15B3 and C38H2, respectively) were injected into unc-69 or unc-69 ced-9/++ animals, and established transgenic lines were tested for rescue of the unc-69 and ced-9 phenotypes. ND: not determined.

Figure 10 shows the ced-9 locus. a) Restriction map. b) Rescue ability of deletions and point mutations in the locus. c) ced-9 transcripts and location of introns and exons.

Detailed Description of the Invention

Programmed cell death is cell death which occurs during normal development and/or which involves the activities of cell death genes, some of which may be suicide genes. Programmed cell death is a fundamental aspect of normal development in invertebrates and vertebrates and of vertebrate tissue homeostasis, and may also be an underlying pathological mechanism in disorders which involve cell death, including degenerative diseases, hair loss, stroke, traumatic brain injury, and myocardial infarction, conditions associated with aging, and viral and other types of

infection. In addition, some cancers are believed to be caused in part by defects in cell death processes.

This invention relates to genes, referred to as cell death-protective genes, which protect cells against programmed cell death by antagonizing the activities of cell death genes (i.e., genes whose activity cause cell death). As described below, a cell death-protective gene from the nematode Caenorhabditis elegans, called ced-9, has been identified, sequenced, and characterized. Mutations which constitutively activate and inactivate ced-9 gene function have been identified and are also described below. As further described below, the deduced amino acid sequence of the ced-9 gene product (SEQ ID NO:3) was found to have about 23% identity and about 47% similarity to the product of the human oncogene bcl-2 (SEQ ID NO:5). The structural and functional similarity of bcl-2 to ced-9 strongly suggests that bcl-2 may be a human equivalent of ced-9 and thus, a cell death-protective gene. Using ced-9 and bcl-2, other cell death-protective genes from a variety of organisms can be obtained. In addition, comparison of equivalent genes and their encoded products, as well as mutational analysis, is expected to indicate key functional features or regions of the genes or gene products. The cell death-protective genes and their gene products are further useful for developing and identifying agents which affect the activity of cell death-protective genes. These agents may be useful for altering (increasing or decreasing) the occurrence of cell death in a cell population or organism, and thus, altering the longevity of the cell population or organism. Further described below are bioassays which are useful for testing and screening for novel cell death-protective genes, mutations in these genes and

agents which affect the activity of the genes. Other uses of the invention are also described.

The activity of a cell death-protective gene refers herein to the activity of the encoded product(s) of the gene as well as to the gene per se. Thus, agents and mutations which affect the activity of a cell death-protective gene include those which affect the activity of the gene or a product of the gene. The agents may interact with the gene or RNA or protein encoded by the gene, or may exert its effect more indirectly. The agents may affect the level of expression as well as the function of the gene or gene product.

Genetic Analyses of the ced-9 Gene

A cell-death protective gene, called ced-9, has been identified in the nematode C. elegans that functions to prevent cells which normally live during development from undergoing programmed cell death. The ced-9 gene was defined by a dominant gain-of-function (gf) mutation, called n1950, which was mapped to chromosome III. The n1950 mutation constitutively activates the ced-9 gene and causes cells which normally die during development to live. Activated ced-9 prevents programmed cell deaths throughout the animal, and, as shown for certain nerve cells, not only prevents cells from dying, but also generates surviving cells that are sufficiently healthy to function. ced-9(n1950) also shows a maternal effect, suggesting that the maternal ced-9 gene product is contributed to the developing oocyte. Genetic analysis of ced-9(n1950) is further described in Example 1 and Table 1 (tables are at the end of the Detailed Description).

Loss-of-function (lf) mutations which inactivate the protective function of ced-9 and cause cells which normally live during C. elegans development to die were

also identified. These mutations result in embryonic lethality in the progeny of homozygous animals, indicating that ced-9 function is essential for development. Four ced-9(lf) mutations were isolated, nDf40, n2077, n2161, and n1653ts. The lf mutations also show maternal effects. The amount of wild-type ced-9 product contributed by heterozygous mothers to homozygous ced-9(lf) embryos seems to be sufficient to allow these embryos to survive and develop almost normally. As a consequence of this maternal rescue, the lethality that results from an absence of ced-9 function during early development is apparent only in the second generation. Most of the ectopic cell deaths observed in the first generation of homozygous ced-9(lf) animals occur late, during post-embryonic development. It is possible that these late lineages are more seriously affected because dilution or degradation has reduced the amount of maternal ced-9 product to a level at which it cannot effectively protect against cell death. The isolation and genetic analysis of these loss-of-function mutations are further described in Examples 2 and 3 and Table 2.

As described in Example 4, the ced-9 gene appears to prevent cell death by antagonizing the activities of the cell death genes, ced-3 and ced-4, which have been shown to be required for almost all programmed cell deaths which occur in the development of C. elegans (Ellis and Horvitz, Cell 44:817-829 (1986)).

These results indicate that ced-9 acts as a binary switch to regulate programmed cell death (Figure 1). Remarkably, it seems that many and possibly all cells that survive during C. elegans development do so because ced-9 gene activity prevents them from undergoing programmed cell death. Furthermore, cells protected by a constitutively activated ced-9 gene appear to be healthy and to function normally. Thus, ced-9 seems to

be a key or master regulatory gene of cell death processes.

Sequence Analysis of the ced-9 Gene and Product

The genomic region containing the ced-9 gene was cloned and sequenced, as described in Example 5. Figure 2 shows the nucleotide sequence (SEQ ID NO:1) of this region, including the location of selected restriction sites.

Several ced-9 cDNAs representing the same or different transcripts were obtained and sequenced, as described in Example 5. The nucleotide sequence (SEQ ID NO:2) of one of these cDNAs is shown in Figure 3 with restriction sites and the amino acid sequence (SEQ ID NO:3) of the predicted translation product. As shown in Figure 4, ced-9 encodes a 280 amino acid (aa) polypeptide.

The gain-of-function mutation, n1950, was also sequenced. As shown in Figure 5, the n1950 mutation, which is responsible for the gain-of-function change in ced-9 activity, is associated with a glycine to glutamic acid change at codon 169. It is likely that this amino acid alteration is the consequence of the n1950 mutation and thus is functionally responsible for the increased activity of ced-9. However, although no other alterations in ced-9 are known to be present in n1950 mutant strains, it remains possible that another alteration exists and that it is this other alteration that is responsible for the gain-of-function change in ced-9 activity. If so, this other amino acid alteration is nonetheless defined by the n1950 mutation and its molecular identity can be determined by DNA sequencing, using established methods. The functional importance of DNA sequence alterations associated with ced-9 mutations can be verified in transgenic C. elegans animals which

carry the sequence alteration alone. DNA containing alterations in the wild-type gene can be made by standard methods of *in vitro* mutagenesis and used to construct the transgenic animals.

The loss-of-function mutations, *n1653ts* and *n2077*, were also sequenced and found to be associated with a tyrosine to asparagine change at codon 149 and a glutamine to premature termination at codon 160, respectively.

Similarity Between ced-9 and a Human Oncogene

Sequence similarity to the *ced-9* gene product (SEQ ID NO:3) was discovered in the product of the human oncogene *bcl-2* (SEQ ID NO:5) (Tsujimoto *et al.*, *Proc. Natl. Acad. Sci. USA* 83:5214-5218 (1986)). Alignment of the two sequences shows 23% identity and 47% similarity between the two proteins (Figure 6). Alignment of the two sequences was generated with the Gap program in the Sequence Analysis Software Package (Genetics Computer Group, Wisconsin), which uses the algorithm of Needleman and Wunsch (*J. Mol. Biol.* 48:443-453 (1970)) to find the alignment of two complete sequences that maximize the number of matches and minimizes the number of gaps.

bcl-2 is one of a number of genes, of both viral and cellular origin, which are thought to be inhibitors of cell death processes (Vaux *et al.*, *Nature* 335:440-442 (1988); Henderson *et al.*, *Cell* 65:1107-1115 (1991); Ciem *et al.*, *Science* 254:1388-1390 (1991)). Overexpression of *bcl-2* prevents or delays the onset of apoptic cell death in both B cells and T cells (Vaux *et al.* (1988) *supra*; Nunez *et al.*, *J. Immun.* 144:3602-3610 (1990); Sentman *et al.*, *Cell* 67:879-888 (1991); Strasser *et al.*, *Cell* 67:889-899 (1991)). These cell deaths seem to involve the activities of cell death genes, as gene expression is required for the process (Duke and Cohen,

Lymphokine Res. 5:289-299 (1986)). In many tissues in which homeostasis is regulated by cell death, bcl-2 expression occurs in progenitor and long-lived cells (Hockenbery et al., Proc. Natl. Acad. Sci. USA 88:6961-6965 (1991)). The structural similarity of the Bcl-2 protein to Ced-9, together with these previous findings on bcl-2 activity in lymphocytes, suggest that, like ced-9, the bcl-2 gene antagonizes the activities of cell death genes and is required in cells that survive to protect them from programmed cell death.

Cell Death-Protective Genes in Other Organisms

As a result of the work described herein, a gene which plays a key role in determining cell death has been identified, sequenced and characterized. This invention makes it possible to identify and isolate equivalent genes in other organisms, including vertebrates (e.g., mammals and particularly humans), invertebrates (e.g., insects), microbes (e.g., yeast), and possibly plants. The reasonableness of this approach has been demonstrated by the structural and functional similarity of the human gene bcl-2 to ced-9. As discussed below, there is evidence to suggest that programmed cell death is important in the development of a variety of organisms and tissues, and that different types of cell deaths, whether physiological or pathological, may share a common mechanism.

Evidence suggests that cell deaths that are mechanistically similar to the programmed cell deaths which occur in the development of C. elegans and other invertebrates may be very common in vertebrate development, as well. First, cell deaths that are similar to the programmed cell death seen in invertebrates were also observed in vertebrates (Glucksman (1951) supra; Saunders and Fallon, in: Major

Problems in Developmental Biology (25th Symposium of the Society for Developmental Biology), Lockes (ed.) Academic Press, New York, 1966, pp. 289-314; Carr and Simpson, Dev. Brain Res. 2:57-162 (1982)). Some of these cells die shortly after they are born without obvious differentiation and others have been shown to be determined to die days before death occurs.

Second, some vertebrate neuronal cell deaths involve cell interactions and have been thought by some to be different from the apparently cell autonomous programmed cell deaths observed in invertebrates. However, even among the cell dependent deaths of these vertebrate neurons, the expression of new genetic information was found to be necessary for cell death, suggesting that a cell death program must be activated. Thus, it is possible that genes similar to those responsible for programmed cell death in C. elegans (ced-3 and ced-4) act in these vertebrate cell deaths as well.

Third, although many cell deaths in invertebrates are invariant and many in vertebrates are variable, the same mechanisms may nonetheless be responsible. Specifically, invariability does not reveal the mechanism of cell death. For example, invariability could be the result of an invariant cell-cell interaction. Thus, the linker cell in C. elegans males always dies at a certain specific time, but its death requires cell-cell interaction. The invariable programmed cell deaths in moths can, in fact, be prevented by manipulating their hormonal environment or changing cell-cell interactions. These observations argue that the distinction between programmed cell death during invertebrate development and cell death during vertebrate development may be more superficial than real. All of these cell deaths are influenced by genetic factors. Such argument leads to the notion that

many and possibly all naturally occurring cell death during development may involve similar mechanisms.

The induction of specific genes has been found during the cell death processes in vertebrates. The induction of TRPM-2 was observed during a variety of cell death processes in rodents (Buttyan et al., Mol. Cell. Biol. 9:3473-3481 (1989)). TRPM-2 RNA is at high levels in cells that die during prostate regression in the adult male rat after castration, during renal atrophy following ureteral obstruction in rat, during necrosis of interdigital tissues of the mouse limb bud, and during the chemotherapeutic regression of tumors in rat. Thymocyte cell deaths induced in response to a variety of stimuli can have very similar morphological and biochemical properties, implying the involvement of a single mechanism of cell death. These observations suggest strongly that many types of cell deaths may share a similar mechanism.

In addition, it is possible that of the many human disorders characterized by extensive cell deaths, such as degenerative diseases, stroke, myocardial infarction and traumatic brain injury (for example, see Choi, Neuron 1:623-634 (1988)), some are caused by processes that inactivate or bypass the functions of genes like bcl-2 and ced-9. Furthermore, intracellular calcium has been implicated as a common mediator in a variety of pathological cell deaths, including deaths caused by external injury, toxins, degenerative diseases, ischemia, and hypoxia (Schanne et al., Science 206:700-702 (1979); Farber, Life Science 29:1289-1295 (1981)). Interestingly, amino acid sequence analysis of the C. elegans ced-4 gene product indicates that the Ced-4 protein may contain calcium-binding domains (Yuan, Ph.D. thesis, Harvard University, 1989, p. 139).

Uses of the Invention

This invention provides agents and methods based on ced-9, bcl-2, and other cell death-protective genes that are useful for diagnosis and treatment (both therapeutic and preventive) of a variety of disorders and conditions involving cell death. The invention is applicable to a variety of organisms, including humans. The genes and their encoded products can be used directly in therapeutics or provide a basis for designing and identifying agents which affect the occurrence of cell death. In addition, mutant forms of these regulatory genes, their encoded products and derivatives of the encoded proteins are available that are potentially useful for treatment.

Other cell death-protective genes can be obtained using the methods provided by this invention. As discussed above, it is likely that genes that are structurally and functionally similar to the C. elegans ced-9 gene function in a variety of organisms, including vertebrates (e.g., mammals and particularly humans), invertebrates (e.g., insects), microbes (e.g., yeast), and possibly plants. These equivalent genes have nucleotide sequences similar to portions of the ced-9 gene, or their encoded products have amino acid sequences similar to portions of the ced-9 protein. Equivalent genes also have similar activity to ced-9, in that they protect the cells in which they function from cell death. For example, the human gene bcl-2 was found to be equivalent to ced-9, as described herein.

Novel cell death-protective genes can be identified by any number of detection methods which utilize a defined nucleotide or amino acid sequence or antibodies as a probe. The genomic (SEQ ID NO:1) and cDNA (SEQ ID NO:2) nucleotide sequences of ced-9 and the deduced amino acid sequence (SEQ ID NO:3) of the Ced-9 protein

are shown in Figures 2, 3, and 4, respectively. The bcl-2 gene and gene products can also be used as probes for cell death-protective genes. The cDNA nucleotide sequence of bcl-2 (SEQ ID NO:4) and the deduced amino acid sequence (SEQ ID NO:5) of the Bcl-2 protein are shown in Figures 6 and 7. For example, nucleic acid (DNA or RNA) containing all or part of the ced-9 or bcl-2 genes can be used as hybridization probes or as polymerase chain reaction (PCR) primers. Degenerate oligonucleotides derived from the amino acid sequences of the ced-9 or bcl-2 gene products can be used in these methods. In addition, antibodies, both polyclonal and monoclonal, which bind specifically to the Ced-9 and/or Bcl-2 protein can be produced and used as immunoprobes to screen expression libraries of genes. Databases containing known molecular (nucleotide or amino acid) sequences can also be searched for molecules which are structurally similar to ced-9, bcl-2, or their encoded products.

One strategy for detecting novel cell death-protective genes in various organisms is to initially probe animals which are taxonomically closely related to the source of the probes, for example, probing other worms with a probe derived from ced-9 or probing other mammals with a probe derived from bcl-2. Closely related species are more likely to possess cell death-protective genes or products which are detected with the probe than are more distantly related organisms. These new genes then provide additional sequences with which to probe the molecules of other animals, some of which may share conserved regions with the new genes or gene products but not with ced-9 or bcl-2. This strategy of using related genes in taxonomically closer organisms as stepping stones to genes in more distantly related organisms can be referred to as walking along the

taxonomic ladder. However, cell death-protective genes or gene products from a variety of organisms may possess considerable sequence similarity and hence, be identifiable by more direct approaches.

The ced-9 and bcl-2 gene products were found to have 23% identity and 47% similarity. The molecular similarity between the ced-9 and bcl-2 gene products is useful, because the similarities between the two proteins reveal which parts or features of these molecules are important for function. For example, an activated bcl-2 may be produced by mutation of the codon which is equivalent to the site of the n1950 mutation in ced-9. More insights on the structure-function relationship of cell death-protective genes are expected to be obtained as more genes equivalent to ced-9 and/or bcl-2 are compared. This knowledge can be used to develop novel molecules which mimic or alter the activity of ced-9, bcl-2 or other cell death-protective genes.

Cell death-protective genes identified as described above can be sequenced by standard methods. Mutated forms of the genes may be identified by such methods, and some of these mutations are expected to constitutively activate and some to inactivate the genes like the n1950 gain-of-function and the loss-of-function mutations in ced-9. Mutationally activated and inactivated forms of cell death-protective genes may be useful for treatment of various disorders, as described further below. In addition, mutagenesis and other sorts of alterations can be performed on the genes and their encoded products to obtain other activated or inactivated proteins.

Mutations may also produce cell death-protective proteins with novel properties. For example, it is conceivable that a cell death-protective gene could be al-

tered such that the gene product actively kills cells, rather than protecting them from cell death, perhaps by activating cell death genes or interfering with the function of wild-type cell death-protective gene products. Mutations and other alterations can be accomplished using known methods, such as in vivo and in vitro mutagenesis.

Furthermore, ced-9, bcl-2, or other cell death-protective genes, the corresponding mutant genes, and encoded products can be used to develop agents that activate or inactivate or modulate the activity of the cell death-protective genes. The source of the agents can be such traditional sources as extracts (e.g., bacterial, fungal or plant) and compound libraries, or can be provided by newer methods of rationale drug design. Information on functionally important regions of the genes or gene products, gained by sequence and/or mutational analysis, as described above, would be useful in drug design. The activity of the agents can be verified both by in vivo tests on wild-type, mutant, or transgenic animals containing various forms of ced-9, bcl-2, or other cell death-protective genes, as described below, and by in vitro tests using either cells expressing such genes or the products of these genes directly in biochemical experiments. Potential agents may include all or portions of the ced-9 or bcl-2 genes or gene products (DNA, protein), all or portions of other cell death-protective genes and their encoded products, nucleic acid or peptide derivatives of cell death-protective genes and gene products (e.g., smaller polypeptides and peptides), as well as peptido-mimetics, and other molecules which mimic or affect the activity of cell death-protective genes. The agents can also be portions or derivatives of genes which do not by

themselves protect cells from programmed cell death but which interact with cell death-protective genes.

This invention further provides bioassays which measure the activity of cell death-protective genes, and hence, are useful for identifying cell death-protective genes, for testing mutations in these genes, and for developing agents which mimic or alter cell death-protective activity. The bioassays can be further used to screen expression gene libraries for novel cell death-protective genes from nematodes and other organisms.

In one bioassay, genes or agents are introduced into nematodes to test their effect on cell deaths *in vivo*. Wild-type, mutant, or transgenic nematodes can be used as appropriate for the expected effect being tested. In one embodiment of the bioassay, transgenic nematodes are produced using sample DNA containing a candidate cell death-protective gene, a mutant cell death-protective gene or a gene library, to observe the effect of the sample DNA on the pattern of cell deaths during development of the nematode, using the methods of genetic analysis described for the *ced-9* mutations. For example, a candidate gene can be introduced into a nematode which has a loss-of-function mutation in *ced-9* to produce a transgenic nematode. A decrease in cell deaths compared to nontransgenic nematodes would indicate that the sample gene has cell death-protective activity. Similarly, a mutant cell death-protective gene which is inactivated would fail to complement the *ced-9* loss-of-function mutation in the transgenic nematode, whereas a constitutively activated gene would decrease the number of cell deaths resulting from the mutation.

In another embodiment of the nematode bioassay, wild-type, mutant, and transgenic nematodes are used to

test the effects of specific peptides and other small molecules in order to identify drugs that mimic, increase or decrease cell deaths. For example, wild-type animals can be used to test agents that inactivate or decrease the activity of ced-9 and cause increased cell deaths, or that activate or increase the activity of ced-9 and decrease or prevent cell deaths. Mutant or transgenic animals in which ced-9 is underexpressed or inactivated could be used to identify agents that mimic ced-9 in preventing cell death or which act as agonists of cell death-protective activity. Likewise, mutant or transgenic animals in which ced-9 is overexpressed or constitutively activated can be used to identify agents which act as antagonists of cell death-protective activity. Nematodes expressing wild-type ced-9 could be used to identify agents which activate or inactivate the ced-9 gene. The agents may include genes which are not cell death-protective genes but which interact with, regulate, or otherwise affect the activity of ced-9. The agents can be introduced into nematodes by microinjection, diffusion, or ingestion.

Furthermore, agents which affect the activity of other cell death-protective genes, such as bcl-2, can be tested by transgenic animals with a loss-of-function mutation in ced-9. Agents which are non-cell death-protective genes can be tested on cell death-protective genes other than ced-9 by constructing doubly transgenic animals. These animals can be made by crossing a transgenic line which expresses a cell death-protective gene and an inactivated ced-9 gene with a transgenic line which expresses the agent gene.

An in vitro bioassay is also provided. In this bioassay, cultured mammaliam cells are used to test genes and agents. Expression gene libraries can also be screened by this method. For example, genes, including

genes which are structurally similar to ced-9 or bcl-2, can be introduced into mammalian cells by standard transfection methods to see if they protect from cell death under conditions which induce cell death, such as exposure to toxins or infection by yeast or bacteria. Mutations which activate or inactivate or otherwise affect cell death-protective activity can be tested. Furthermore, transfected mammalian cells which express a wild-type or mutant cell death-protective gene can be used to test agents which increase or decrease the activity of cell death-protective genes.

Using the above-described nucleic acid and antibody probes and bioassays, the identification and expression of ced-9, bcl-2 or other cell death-protective genes in cultured cells, tissues, and whole organisms can be studied to gain insights into their role in development and pathology. For example, these methods of detection and bioassay can be used to determine if certain mutations in cell death-protective genes, such as bcl-2, are associated with a pathological condition, such as a degenerative disorder.

This invention further provides means of altering or controlling the activity of a cell death-protective gene in a cell or organism, and, thus, to affect the occurrence of cell death. Activity of the regulatory gene can be altered to either increase or decrease cells deaths in a population of cells and, thus, affect the proliferative capacity and/or longevity of a cell population, organ, or entire organism.

ced-9, bcl-2, or other cell death-protective genes, and related and derivative products can be used to protect against cell death of any sort, including degenerative disease, stroke, traumatic brain injury, myocardial infarction, and viral and other types of infection, as well as cell death associated with normal

aging. The gene, its encoded RNA, the protein encoded by the gene, or a peptide derived from or related to the gene can be delivered to the affected cells by various methods appropriate for the cells or organs being treated, including gene therapy. A non-peptide molecule which mimics, activates, or enhances the activity of a protein encoded by ced-9 or other cell death-protective gene, or polypeptide or peptide derivative, and which is designed on the basis of knowledge of the encoded protein, can also be used. That is, the gene or its product may be used either directly to protect against cell death or as the basis for developing another agent that can function like or increase the activity of the gene or its encoded product.

Mutationally activated forms of the genes can also be used to protect against cell death. Again, the mutated gene, its encoded RNA, the mutant protein encoded by the gene, a peptide derived from or related to the mutant protein, or a non-peptide mimetic, activator or agonist can be used. The n1950 mutation in ced-9 defines one way to make such a gene activated. A mutation equivalent to n1950 can be placed in a cell death-protective gene similar to ced-9 to activate it. For example, a constitutively activated bcl-2 protein might be produced by making a glycine to glutamic acid change at codon 145, as shown in Figure 6, or other sequence alteration equivalent to the one which is responsible for the phenotype of the n1950 mutation in ced-9. (It has not yet been definitively shown that the glycine to glutamic acid alteration of codon 169 of ced-9 is responsible for the activated phenotype of the n1950 mutation. If it is not, the other mutational change(s) in ced-9 responsible for the activation of this gene can be identified as described above and produced in bcl-2 by in vitro mutagenesis to activate

bcl-2). The mutant Bcl-2 protein may then be used as a clinically useful molecule or as a basis for developing or identifying a clinically useful molecule which protects from cell death.

Alternatively, ced-9, bcl-2, or other cell death-protective genes and their encoded products can be inactivated, or their activity reduced, in order to increase the frequency of cell death. This would be useful, for treating diseases and conditions characterized by an abnormally low frequency of cell death or excessive cell growth, such as neoplastic growth and other cancers. Interestingly, the human cell death-protective gene bcl-2 is also an oncogene, suggesting that cell death processes can be affected in neoplasia. Methods and agents which increase cell death would also be useful for decreasing the growth of or eliminating specific cell populations. For example, populations of autoreactive immune cells may be eliminated or reduced for treating autoimmune disorders. The activity of bcl-2 or other equivalent cell death-protective gene may be inactivated by using single stranded nucleic acid having an antisense sequence which is complementary to the normal transcript of the cell death-protective gene, such as antisense RNA, or DNAs which encode the antisense nucleic acid, or inactivators or antagonists of cell death-protective activity. These agents can be delivered by a variety of methods, including gene therapy. Inactivation of cell death-protective genes may also be useful in treating viral, parasitic and other types of infection, such as human immuno-deficiency virus (HIV) infection. A recombinant gene encoding an inactivator or antagonist of cell death-protective activity, such as antisense RNA which is complementary to the transcript of a cell death-protective gene, may be linked to a viral promoter which

is specifically activated by a viral protein. The recombinant gene is introduced into infected cells. Infected cells containing the viral protein would then be killed and uninfected cells would be unaffected.

Inactivation of cell death-protective genes may also be used to kill organisms for the purpose of biological containment, pest control, or other applications in which populations of undesirable organisms are to be reduced. For example, suicide genes used for biological containment of recombinant bacteria have been reported (Genetic Engineering News, Nov. 1991, p. 13). The suicide genes were engineered to be expressed simultaneously with the desired recombinant gene product so that the recombinant bacteria die upon completion of their task. The present invention provides for construction of recombinant suicide genes encoding antisense RNAs or other inactivators or antagonists of ced-9 or other cell death-protective genes which are useful in organisms in addition to bacteria, for example, in insects, fungi, and transgenic rodents.

Agents which inactivate or inhibit cell death-protective genes can further be used for pest control. For example, many nematodes are human, animal, or plant parasites. Populations of such parasites could be reduced or eliminated by causing their cells to undergo programmed cell death. Parasites present in host animals, including humans, may also be reduced by treatment with agents, such as antisense RNAs, which decrease the activity of a cell death-protective gene specific to the parasite and which leave the host animal unharmed.

The following examples illustrate the invention and are not intended to be limiting in any way.

EXAMPLE 1

Gain-of-Function Mutation in ced-9

While screening for new C. elegans mutations that affect programmed cell death (Ellis and Horvitz, Development 112:591-603 (1991)), a dominant mutation, n1950, was isolated and genetically characterized, that prevents programmed cell deaths. n1950 was mapped to the right arm of the third chromosome, close to and about 0.05 map units to the right of the mutation unc-69(e587). The n1950 mutation defines a new gene, ced-9 III.

To quantify the effects of the ced-9(n1950) mutation on programmed cell deaths, cells in the anterior half of the pharynx of ced-9(n1950) animals were counted. In wild-type animals there are 49 cell nuclei in this region (Sulston et al., Devl. Biol. 100:64-119 (1983); Albertson and Thomson, Phil. Trans. R. Soc. B275:299-325 (1976)), and in ced-3 and ced-4 animals there are 12-14 additional nuclei (Table 1a). Similarly, in ced-9(n1950) animals there are about 13 extra nuclei in the anterior pharynx. These extra nuclei correspond exactly in position as well as in number to those that fail to die in ced-3 and ced-4 mutants.

Many extra cells survive not only in ced-9(n1950) homozygotes but also in ced-9(n1950)/+ heterozygotes, indicating that the n1950 phenotype is dominant (Table 1b). In addition, the ced-9(n1950) mutation has a maternal effect: about twice as many cells fail to die in heterozygotes generated by mothers carrying at least one copy of the ced-9(n1950) mutation than in heterozygotes generated by homozygous wild-type mothers (Table 1b), suggesting that maternal ced-9 gene product is contributed to the developing oocyte.

Two observations indicate that ced-9(n1950) is a gain-of-function (gf) mutation. First, n1950 is a rare mutation with dominant effects (only one allele was recovered in a screen of over 24,000 haploid genomes (Ellis and Horvitz (1991) supra), which is a frequency about 10-fold lower than that at which typical loss-of-function mutations are recovered (Brenner, Genetics 77:71-94 (1974); Meneely and Herman, Genetics 92:99-115 (1979); Greenwald and Horvitz, Genetics 96:147-164 (1980)). Second, a deletion (nDf40) that removes the ced-9 gene does not have a dominant effect on cell death (Table 1b).

To study the effects of ced-9(n1950) on programmed cell deaths in regions other than the anterior pharynx, it was determined whether n1950 could prevent the accumulation of cell corpses in ced-1 and ced-5 mutants. In wild-type animals, dying cells are rapidly engulfed and degraded by a neighboring cell. In ced-1 and ced-5 mutants, this engulfment process is blocked, leading to an accumulation of undegraded cell corpses that can easily be seen in young larvae (Hedgecock et al., Science 220:1277-1279 (1983); Ellis et al. Genetics 129:79-94 (1991)). Mutations that inactivate ced-3 or ced-4 block programmed cell death and therefore prevent the accumulation of dead cells in ced-1 or ced-5 animals (Ellis and Horvitz, Cell 44:817-829 (1986)). Similarly, very few corpses appear anywhere in ced-1; ced-9(n1950) or ced-9(n1950); ced-5 double mutants (Table 1c). Thus, the ced-9(n1950) mutation, like mutations in ced-3 and ced-4, prevents programmed cell deaths throughout the animal.

The effects of ced-9(n1950) on the survival and function of a specific pair of nerve cells, the HSNs (hermaphrodite-specific neurons) were also studied (Trent et al., Genetics 104:619-647 (1983); White et

al., Phil. Trans. R. Soc. B311:1-340 (1986); Desai et al., Nature 336:638-646 (1988); Desai and Horvitz, Genetics 121:703-721 (1989)). The two HSN neurons innervate the vulval muscles and control egg-laying by hermaphrodites. Mutations in the gene egl-1 cause these cells to undergo programmed cell death, resulting in egg-laying defective animals (Ellis and Horvitz, Cell 44:817-829 (1986); Trent et al., (1983) supra; Desai and Horvitz (1989) supra). Mutations in ced-3 and ced-4, which block programmed cell death, prevent the HSNs from dying in egl-1 mutants and suppress the egg-laying defect (Ellis and Horvitz (1986) supra). Similarly, the HSNs are present in ced-9(n1950); egl-1 double mutants, and egg-laying by these animals is normal (Table 1d). Thus, ced-9(n1950), like the ced-3 and ced-4 mutations, not only prevents cells from dying but, at least in this case, also generates surviving cells that are sufficiently healthy to function.

Methods

The data presented in Table 1 were obtained as follows. Cell survival was quantified by counting the cells in the procorpus and metacorpus, which together constitute the anterior half of the pharynx (Albertson and Thomson, Phil. Trans. R. Soc. B275:299-325 (1976)). In wild-type animals there are 49 cell nuclei in this region. Cells that die are generated in characteristic positions (Sulston et al., Devl. Biol. 100:64-119 (1983)), making it easy to identify and count cells that have failed to die. The genotypes of animals studied for Table 1a were as shown.

The complete genotypes of the animals studied for Table 1b were, from top to bottom: wild-type (N2), non-Unc progeny of eT1 unc-36/nDf40 dpy-18 males crossed with unc-36 hermaphrodites, non-Unc progeny of n1950

males crossed with unc-69 hermaphrodites, Unc Dpy progeny from n1950/unc-69 dpy-18 hermaphrodites, non-Lon non-Dpy progeny from dpy-17 lon-1/n1950 dpy-18 hermaphrodites, Unc-49 progeny from unc-69/n1950 unc-49 heterozygous hermaphrodites, non-Unc progeny of wild-type (N2) males crossed with unc-69 n1950 hermaphrodites, and n1950 self-progeny from n1950 homozygous hermaphrodites.

For the pharyngeal and head corpses in Table 1c, only young larvae with four cells in the gonad, that is, between hatching and the middle of the first larval stage, were scored (Kimble and Hirsh, Dev. Biol. 70:396-417 (1979)). For ventral cord corpses (descendants from the blast cells P9-P12) and for tail corpses, third larval stage animals were scored. Extra cells are the number of extra cells among the descendants of P9, P10, and P11. The divisions of these blast cells generate four programmed cell deaths in the wild-type (Figure 6a).

In Table 1d, HSN missing (%) is the percent of missing or grossly displaced HSN neurons. Only first or second larval stage animals were scored. There are two HSNs per animal, one on each side (White et al., Phil. Trans. R. Soc. B311:1-340 (1986)). To score egg laying, staged worms were grown at 20°C. Animals were observed using a dissecting microscope on the second day of adulthood, and those bloated with late-stage eggs were considered egg-laying-defective (Trent and Horvitz, Genetics 104:619-647 (1983)). The alleles used were: ced-1(e1735), ced-3(n717), ced-4(n1162), ced-5(n1812), ced-9(n1950), dpy-17(e164), dpy-18(e364), egl-1(n478sd, ts), lon-1(e1820), unc-36(e251). eT1(e873), a translocation chromosome with a breakpoint that disrupts unc-36 gene function, prevents crossing over on the right arm of chromosome III (Rosenbluth and Baillie,

Genetics 99:415-428 (1981)). nDf40 is a new deficiency which was isolated as a cis-acting suppressor of n1950.

Animals were anaesthetized with 30 mM NaN_3 (Avery and Horvitz, Cell 51:1071-1078 (1987)) and observed using Nomarski optics microscopy (Sulston and Horvitz, Devl. Biol. 56:110-156 (1987)). Average numbers are shown with, if appropriate, their 95% confidence limits, as determined by the t-test using the StatViewII program (Abacus Concepts, Berkeley, California).

EXAMPLE 2
Isolation of ced-9(lf) Mutations

Because the ced-9(n1950) mutation causes a gain of gene function (see above), mutations that reduce or eliminate ced-9 activity (ced-9 loss-of-function (lf) mutations) were isolated by screening for cis-dominant suppressors of ced-9(n1950). Second mutations in ced-9 were expected to be isolated which could suppress the dominant effects of n1950 by inactivating ced-9 (Figure 7). After screening 9,000 haploid genomes, three candidate suppressor mutations were isolated which were tightly linked to ced-9(n1950). One of these mutations, nDf40, behaved genetically as a large deletion (see below), indicating that the screening procedure should allow the isolation of mutations that completely inactivate ced-9. The other two mutations, n2077 and n2161, seem likely to be ced-9 loss-of-function alleles: these two mutations failed to complement each other while complementing recessive mutations in all known genes in this region. The n2077 and n2161 mutations mapped within 0.1 map units of the original n1950 mutation, and were obtained at a frequency of about 3×10^{-4} per haploid genome, which is comparable to that for loss-of-function mutations in other C. elegans genes

(Brenner, Genetics 77:71-94 (1974); Meneely and Herman, Genetics 92:99-115 (1979); Greenwald and Horvitz, Genetics 96:147-164 (1980)).

It was then determined that another mutation, n1653ts, which was previously isolated in an unrelated screen for mutants with displaced or missing HSN neurons (Desai et al., Nature 336:638-646 (1988)), was also a ced-9(lf) allele. n1653 was shown to be allelic to n2077 and n2161 based on its position on the genetic map, the similarity of its phenotype at restrictive temperature to the phenotypes of n2077 and n2161 mutants, and its failure to complement n2077 and n2161. Programmed cell deaths occurred normally in n1950 n2077/++ and n1950 n2161/++ animals, but were blocked in n1653/n1950 trans-heterozygotes. The results of this cis-trans test demonstrate that the allelic mutations n2077, n2161 and n1653 are in the ced-9 gene, which is defined by the mutation n1950, rather than in a closely linked gene.

Methods

The screen for mutations that resulted in a loss of ced-9 function (see Figure 7) was performed as follows. The semidominant mutation egl-1(n487sd) causes the two HSN neurons to die by programmed cell death, so that the animal bloats with eggs (Ellis and Horvitz, Cell 44:817-829 (1986); Trent et al., Genetics 104:619-647 (1983). Because ced-9(n1950) dominantly suppresses egl-1(n487) by preventing the deaths of the HSN neurons, only animals that do not have ced-9(n1950) function will bloat with eggs as a result of the egl-1 mutation. Such egg-laying defective animals were screened by mating egl-1(n487) V males either with unc-69(e587) ced-9(n1950) III; unc-10(e102) xol-1(y9) dpy-6(e14) X hermaphrodites or with unc-69(e587) ced-9(n1950) dpy-

18(e364) III; lon2(e678) xol-1(y70) X hermaphrodites. Egg laying-defective cross-progeny were picked and their progeny examined for any unusual phenotype. The xol-1 mutation on the X chromosome causes male lethality (Miller et al., Cell 55:167-183 (1988) and so prevents mating among F_1 animals, which would complicate genetic analysis of new mutations. The unc-69, dpy-18, lon-2, unc-10, and dpy-6 mutations were used as closely linked genetic markers to identify the chromosomes carrying the ced-9 and xol-1 mutations. General genetic methods and techniques for mutagenesis with ethyl methanesulphonate are described in Brenner, Genetics 77:71-94 (1974). Two-factor mapping experiments showed the new mutations n2077 and n2161 to be tightly linked to ced-9(n1950). This screen also generated nDf40, a deficiency that fails to complement unc-69, ced-9, unc-49 and several adjoining genes. The loss-of-function mutation ced-9(n1950 n2077) complements the nearby mutations unc-50(e306), ooc-4(e2078) and emb-25(g45ts); ced-9(n1653ts) complements unc-69(e587). The ooc-4 mutation causes a defect in oogenesis, resulting in hermaphrodite sterility. The mutation emb-25(g45ts) is described in Cassada et al., Dev. Biol. 84:103-205 (1981). All other mutations are described in Brenner (1974) supra.

EXAMPLE 3

The ced-9(lf) Alleles Cause Ectopic Cell Deaths

Animals homozygous for ced-9(lf) mutations show several defects (Table 2). Most obviously, homozygous ced-9(lf) mutants derived from ced-9(lf)/+ heterozygous mothers hatch and grow to normal size, but generate very few eggs (partial sterility), all of which eventually die, usually during embryogenesis (maternal effect lethality). Furthermore, such first generation ced-

ced-9(lf) animals lack many cells normally present in wild-type animals, resulting in a number of additional defects. For example, many ventral cord motor neurons involved in the control of movement are missing, resulting in uncoordinated body movement. The HSN neurons are missing in hermaphrodites, causing an egg-laying defect (Table 2). Similarly, cells are absent from the male tail, resulting in missing or deformed rays (Table 2). Furthermore, several neurons are sometimes missing from the lumbar ganglion, although their absence does not result in an obvious behavioral abnormality.

To determine why cells are missing, ced-9(lf) animals were observed as they developed. The pattern of cell divisions in wild-type C. elegans is highly reproducible among individuals, and deviations from the normal cell lineage can be identified (Sulston and Horvitz, Dev. Biol. 56:110-156 (1977); Sulston et al., Dev. Bio. 100:64-119 (1983); Kimble and Hirsh, Dev. Biol. 70:396-417 (1979); Sulston et al., Devl. Biol. 78:542-576 (1980)). The studies revealed that many cells that normally survive in wild-type animals instead undergo programmed cell death in ced-9(lf) animals.

For example, the divisions of the 12 ventral cord blast cells P1-P12 (collectively called Pn) were monitored. During the first larval (L1) stage, each P cell divides to generate an anterior daughter (Pn.a) that is a neuroblast and a posterior daughter (Pn.p) that is a hypodermal blast cell. The Pn.a cells then follow identical patterns of divisions to generate motor neurons involved in locomotion (Sulston and Horvitz (1977) supra; White et al., Phil. Trans. R. Soc. 311:1-340 (1986); Chalfie and White, in: The Nematode Caenorhabditis elegans (eds. Wood et al.), pp.337-391 Cold Spring Harbor Laboratory Press, New York, 1988).

Numerous ectopic cell deaths were observed in all Pn.a lineages of ced-9(lf) animals, and frequently all descendants of the Pn.a neuroblasts died.

Ectopic programmed cell death was also observed in the ray lineages. Rays are simple sensory structures located in the male tail, which is used for copulation. Each of the 18 rays arises from a single ray precursor cell (Sulston and Horvitz (1977) supra). Many ectopic cell deaths occurred in the ray lineages of ced-9(lf) males. These ectopic deaths often eliminated the ray structure cell, which is required for ray formation (Sulston and Horvitz (1977) supra). Thus, these deaths account for the absence of rays in ced-9(lf) males.

To determine the cause of the maternal-effect lethality of ced-9(lf) mutations, the embryonic cell lineages of the progeny of ced-9(lf) animals were studied. Embryos generated by mothers homozygous for the weak allele ced-9(n1950 n2161) usually arrested during the early stages of embryo elongation (about 450 minutes after fertilization; Sulston et al. (1983) supra), although there was some variation from animal to animal. These embryos developed normally to about the 200-cell stage, at which point extensive ectopic cell deaths began to appear. These ectopic cell deaths were morphologically similar to the cell deaths that occur during normal C. elegans development and that also first appear at this stage (Sulston et al. (1983) supra). The cell lineage of a single ced-9(n1950 n2161) embryo was analyzed using a 'four-dimensional'-microscope (which allows time-lapse recording of multiple focal planes of a specimen. 49 of the cells that died (more than 100 cells died eventually) were identified. Of these 49 dying cells, 45 normally survive in the wild-type. These 45 ectopic deaths prevented the generation of 78 cells, 50 of which would have been neurons or glial

cells. Mothers of hypodermal cells and of muscle cells, also died. No obvious pattern to the ectopic cell deaths could be discerned. Many of these deaths involved cells that in the wild-type do not generate any descendants that die. Therefore, these deaths were not simply consequences of premature activation of the pathway for programmed cell death.

Embryos from mothers homozygous for the strong allele ced-9(n1950 n2077) were also studied. The n2077 mutation probably results in a complete or nearly complete inactivation of ced-9, because n2077 behaves like the ced-9 deletion nDf40 when placed in trans to each of the ced-9 alleles (Table 2). Surprisingly, the defects and terminal phenotype associated with this allele were quite different from those of n1950 n2161 embryos. The F_2 n1950 n2077 embryos arrested much earlier in development, with different individuals having from a few dozen to a few hundred cells at most. The embryos invariably looked sick, with swollen cells and abnormal granules in the cytoplasm. Furthermore, cell divisions were slow and asynchronous. In those rare animals that developed sufficiently far before arresting, cell corpses started to appear at about the same stage as in n1950 n2161 embryos. The lineage of a single ced-9(n1950 n2077) embryo was followed with the four-dimensional-microscope. This embryo arrested with 57 cells. Nothing resembling a programmed cell death was observed. However, blocks in mitosis and cytokinesis were apparent, with incomplete cytokineses resulting in the formation of several binucleate cells. It is not known whether these defects in cell divisions and the general sickness are effects of a lack of ced-9 function in the embryo or are secondary consequences of abnormalities in the maternal germline. Because all of these defects are completely suppressed by mutations in

ced-3 or ced-4 (see below), it seems likely that they are a consequence of the ced-9(n1950 n2077) allele rather than of another mutation carried in this strain. It is likely that these defects are caused by the ectopic activation of the pathway for programmed cell death in the maternal germline. Alternatively, the three genes ced-9, ced-3 and ced-4 might act not only in programmed cell death but also in an aspect of early C. elegans development that is unrelated to programmed cell death.

Methods

The loss of ced-9 function results in ectopic cell deaths. Cell lineages of the ventral cord blast cells P1-P12 in the wild-type (Sulston and Horvitz, Dev. Biol. 56:110-156 (1977)) and cell lineages of P1-P12 in a ced-9(n1950 n2077) hermaphrodite progeny of a qC1/unc-69 ced-9(n1950 n2077) heterozygous mother were studied. The exact pattern of cell deaths varied slightly among the three mutant animals studied. Ray lineages in the wild-type (Sulston and Horvitz (1977) supra; Sulston et al., Dev. Biol. 78:542-576 (1980)) were examined, as well as cell lineages of the left and right R cells, R4L-R9L and R4R-R9R, respectively, in a single ced-9(n1950 n2077) male progeny of a qC1/unc-69 ced-9(n1950 n2077) heterozygous mother crossed with males of identical genotype. The left and right R1-R3 cell lineages were not followed in this particular animal. As in the ventral cord lineages, the exact pattern of ectopic cell deaths varied among the three mutant animals studied. Also examined were male tails. Unc-69 male tails had nine rays on each side. The male tail of a particular unc-69 ced-9(n1950 n2077) animal has only three rays on the left side and five on the right side. A ced-4 unc-69 ced-9(n1950 n2077) male tail showed 18 rays. In

wild-type embryos, 350 minutes after fertilization, only a few cell corpses could be seen in ventral view. In ced-9(n1950 n2161) embryos, generated by a ced-9(n1950 n2161) mother, many corpses could be seen 350 minutes after fertilization.

Cell lineages were followed using Nomarski optics microscopy (Sulston and Horvitz (1977) supra). Four-dimensional-microscopy of embryos was done as follows: freshly fertilized embryos were mounted for observation on 5% agar pads in a drop of M9 or egg salts (Sulston et al., Dev. Biol. 100:64-119 (1983)). Pictures of the developing embryos were taken in 18 focal planes (roughly 1 μm apart) at 30 second intervals using an apparatus developed by J. G. White and stored on a 12-inch optical video disk for subsequent analysis.

The data for Table 2 were obtained as follows. For Table 2a, the numbers of eggs laid by first generation ced-9(lf) hermaphrodites and the stages of development at which the progeny of these hermaphrodites arrested were examined. First generation hermaphrodites were transferred to fresh plates every 12 hours, and the number of eggs they laid were counted. Note that the absence of HSNs retards but does not prevent egg-laying (Trent et al., Genetics 104:619-647 (1983)), so that the sterility observed in ced-9(lf) animals as reflected by the number of eggs laid per animal cannot be only an effect of the defect in egg release. For example, egl-1(n487sd, ts) animals, which are egg laying-defective because they lack HSNs, nonetheless lay an average of 204 eggs (Desai and Horvitz, Genetics 121:703-721 (1989)). Egg-laying defective ced-9(lf) animals do, however, fertilize a few eggs that are never laid. For example, although wild-type hermaphrodites lay all fertilized eggs within 4 days of reaching adulthood, by the seventh day ced-9(n1950 n2077) hermaphrodites still

had 1.7±1.3 eggs (number of broods = 12) remaining in utero, and egg-laying defective ced-9(n1950 n2161) hermaphrodites had 30±23 eggs (number of broods = 6). The number of eggs laid by ced-9(lf) animals therefore usually slightly underestimates actual brood size. The percent of eggs laid that hatched within 48 hours of removal of the mother were examined; wild-type eggs hatch about 14 hours after fertilization (Sulston and Horvitz, Devl. Biol. 100:64-119 (1983)). The percent of hatched progeny that failed to develop past the first (L1) larval stage within 6 days of hatching were also examined; wild-type larvae remain in the L1 stage for about 12 hours (Sulston and Horvitz, Devl. Biol. 100:64-119 (1983)).

For Table 2b, the percent of animals defective in egg-laying was scored as in Table 1. Note however that for some genotypes (marked †) a significant fraction of the animals could not be scored accurately for egg-laying capability because of the small numaber of eggs they generated. Egg-laying defective ced-9(lf) animals do lay eggs in the presence of serotonin (assayed as in Trent et al., Genetics 104:619-647 (1983)), suggesting that the serotonergic HSN neurons are defective or absent.

For Table 2c, young adult males were anaesthetized in 30 mM NaN_3, placed on their backs and observed using Nomarski optics. All strains were homozygous (nDf40 strains were hemizygous) for the closely linked mutation unc-69(e587), which facilitates identification of the chromosome carrying the ced-9 mutation. All ced-9(lf) were maintained as heterozygous stocks balanced by the chromosome III balancer gC1. The nDf40 chromosome was marked with dpy-18(e364). nDf40 fails to complement both ced-9 and unc-69. For the n2161/n2077 and n1653/n2077 trans-heterozygotes, the maternally-

inherited n2077 chromosome was marked with the dpy-18(e364) mutation to distinguish self- from cross-progeny. The HSN counts for the n2161/n2077 and n1653/n2077 genotypes were not determined because of the difficulty of scoring the Dpy phenotype in early larvae. The ced-9(+)/Df larvae that arrested as L1s did so as a consequence of the unc-69(e587) mutation, which decreases brood size and results in an incompletely penetrant L1-arrest phenotype when heterozygous with nDf40; by contrast, nDf40/unc-69(+) animals do not arrest development as L1 larvae.

EXAMPLE 4

The ced-9 Gene Antagonizes ced-3 and ced-4

If the defects associated with a loss of ced-9 function are caused entirely by the aberrant activation of the programmed cell death pathway, then mutations that prevent the process of programmed cell death might be able to suppress these defects. To test this hypothesis, double mutants were constructed using ced-9(n1950 n2077) and mutations in either ced-3 or ced-4, two genes required for programmed cell death (Ellis and Horvitz, Cell 44:817-829 (1986)). Mutations in ced-3 or ced-4 completely suppressed all defects observed in ced-9(n1950 n2077) animals (Table 3, Figure 8g). Similar results were obtained for ced-9(n1950 n2161) and ced-9(n1653ts). These observations suggest that the defects seen in ced-9(lf) animals are indeed caused by the activation of the programmed cell death pathway. Furthermore, if these three genes are part of a regulatory pathway, these results indicate that ced-9 acts before ced-3 and ced-4, because the activities of these genes are required for ced-9(lf) mutations to have their effects.

Methods

The data for Table 3 were obtained as follows. The numbers of eggs laid were determined as described for Table 2. Viable progeny are the number of progeny that grew to the fourth larval (L4) stage within 10 days of hatching (this value includes a few animals that developed from eggs that hatched internally); wild-type larvae reach the L4 stage within 2 days. (Sulston and Horvitz, Devl. Biol. 56:110-156 (1977)). Confidence limits (95%) were determined as in Table 1. Note that ced-3 and ced-4 are able to suppress the ced-9(lf) zygotic defects in a semidominant fashion: animals homozygous for ced-9(n1950 n2077) but carrying only one wild-type copy of either the ced-3 of ced-4 genes showed milder zygotic defects than did animals with two wild-type copies of both genes, suggesting that lowering ced-3 or ced-4 activity can compensate for lower levels of the Ced-9 protein in first generation ced-9(lf) animals. However, one copy of ced-3 or ced-4 is not sufficient to suppress the maternal-effect lethality: all the viable progeny generated from ced-9(lf); ced-3/+ mothers were homozygous for the ced-3 mutation. Double mutants were also constructed between ced-9(n1950 n2077) and ced-3(n718), ced-3(n1040), ced-3(n1128), ced-3(n1949), ced-4(n1894), or ced-4(n1920), and between ced-9(n1950 n2161) and ced-3(n717), ced-4(n1162), ced-4(n1894), or ced-4(n1920). All of these double mutants were both viable and fertile, showing that the suppression of the ced-9(lf) defects by ced-3 and ced-4 is not allele-specific.

EXAMPLE 5

Cloning and Sequencing the ced-9 Gene and cDNA

Cloning of ced-9

ced-9 was genetically mapped to the right arm of chromosome III, approximately 0.05 map units to the right of unc-69. This position placed ced-9 between the two cloned genes lin-12 and tra-1. The whole interval between these two genes, corresponding to approximately 2 Mb, had previously been cloned as part of the C. elegans physical mapping effort (Coulson et al., Proc. Natl. Acad. Sci. USA 85:4397-4401 (1986); Coulson et al., Nature 335:184-186 (1988); Coulson et al., BioEssays 13:413-417 (1991)). To narrow down the region containing ced-9, ced-9 was mapped with respect to a series of restriction fragment length polymorphisms (RFLPs) between the common laboratory strain Bristol N2, and RC301, a strain isolated from the wild near Freiburg, Germany.

Mapping of ced-9 with respect to these RFLPs (Figure 9b) localized the gene to a roughly 60 kb interval located between nP55 and nP56. Cosmids from this region were then tested for their ability to rescue the ced-9(lf)-associated defects. Three overlapping cosmids were found to be able to rescue ced-9 (Figure 9c). Various fragments from the region common to all three cosmids were subcloned into plasmids and tested for rescue activity. A 4.7 kb SacI-XhoI fragment was identified in this manner that efficiently rescued both the sterility and the lethality of ced-9(n1950 n2077) mutants. Further deletions into this fragment from either the right or the left abolished or greatly diminished the rescuing the activity of the fragment (Figure 10b).

ced-9 cDNAs

A 4.2 kb ClaI-XhoI fragment was used to probe a C. elegans cDNA library. Three distinct classes of cDNAs were isolated, corresponding to the 0.75 kb, 1.3 kb, and 2.2 kb transcripts identified on Northern blots. One cDNA of each class was sequenced. The deduced intron/exon structure of the three classes of cDNAs is shown in Figure 10c. Both the 0.75 kb and 2.3 kb cDNAs sequenced had SL1 trans-spliced leaders at their 5′ ends, suggesting that full-length cDNAs were isolated. The three cDNA classes are related to each other in an unusual way: the 0.75 and 2.2 kb transcripts share the same 5′ end and open reading frame and are predicted to encode identical proteins. The 1.3 kb and 2.2 kb transcripts are predicted to share the same polyadenylation site.

METHODS:

Mutations and Strains

All mutations were generated in a Bristol N2 background, which was used as the standard wild-type strain, except where noted. The following mutations were used:

LGIII: unc-69(e587), ced-9(n1950dm), ced-9(n1950 n2077), ced-9(n1950 n2161), unc-49(e382).

Mutations other than ced-9 are described in (Brenner, Genetics 77:71-94 (1974)). The ced-9(n1950 n2077) and ced-9(n1950 n2161) mutations were maintained as balanced strains over the LGIII balancer qC1, which carries the mutations dpy-19(e1259ts,mat) glp-1(q339) Strains were maintained as described (Brenner, 1974 supra). All strains were grown at 20°C.

RFLP Mapping

Various cosmids from the lin-12 to tra-1 interval were tested for their ability to detect RFLPs between the common laboratory strain N2 and various strains isolated from the wild and known to contain a large number of transposon-induced RFLPs. The position of ced-9 was then determined relative to these markers as described (Ruvkun et al., Genetics 121:501-516 (1989)). Briefly, N2/RC301 recombinants in the ced-9 region were obtained by mating RC301 males with unc-69(e587) ced-9(n1950) unc-49(e382) hermaphrodites to generate unc-69 ced-9 unc-49 [N2]/+++[RC301] heterozygotes. From these heterozygotes, Unc-49 non-Ced-9 non-Unc-69 and Unc-49 Ced-9 non-Unc-69 recombinants were cloned, homozygozed for the recombinant chromosome, and the genotype of the various RFLP loci analyzed by genomic Southern blot analyses.

Germline Transformation of ced-9 Mutants

DNAs to be tested for ced-9 rescue activity were microinjected into the mitotic germline of hermaphrodites according to the method developed by Mello and colleagues (Mello et al., EMBO J. 10:3959-3970 (1991)). The relevant DNA was injected at a concentration of 5-25 μg/ml. pRF4, a plasmid containing a dominant rol-6 mutation, was co-injected as a dominant marker to identify transgenic animals. Since ced-9(lf) mutants are almost sterile and produce only dead progeny, heterozygotes of genotype qC1 dpy-19(e1259)/unc-69(e587) ced-9(n1950 2077) were injected, where the unc-69 was used as a linked marker to identify the ced-9 chromosome. Non-Unc non-Dpy Roller F1s were picked to establish stably transmitting roller lines. From these, Roller Unc-69 animals were picked and tested for rescue of the ced-9(lf)-associated sterility and

maternal effect lethality. A clone was considered to rescue if a stable homozygous line of genotype unc-69 ced-9(lf) III; array could be established.

Molecular Biology

Standard molecular biology protocols (see (Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, 1989) was followed except where noted. All plasmid subcloning was done into pBluescript vectors (Stratagene).

The 4.7 kb genomic SacI-XhoI fragment with rescuing ability was subcloned into pBluescript II (Stratagene) and both strands were sequenced using the ExoIII-S1 nested deletion method and T7 polymerase (Sequenase, USB) following the protocol suggested by the manufacturer.

A 4.2 kb ClaI-XhoI rescuing genomic fragment was used to probe a λ cDNA library. From approximately 300,000 plaques, 11 cDNAs were isolated. The sequences present at the ends of the inserts were determined for all 11 cDNAs. 8 cDNAs corresponded to the 0.75 kb cytochrome b560-like transcript, two (one incomplete) were from the 1.3 kb ced-9 transcript, and one corresponded to the 2.2 kb "fusion" transcript. One cDNA from each class was then completely sequenced (one strand only).

TABLE 1

The Gain-of-Function Allele *ced-9(n1950)* Prevents Programmed Cell Deaths

(a) *ced-9(n1950)* prevents programmed cell deaths

Maternal genotype	Zygotic genotype	Extra cells in anterior pharynx	No. of Animals
ced-3/ced-3	*ced-3/ced-3*	12.5±0.7	30
ced-4/ced-4	*ced-4/ced-4*	13.9±0.5	40
ced-9(n1950)/ced-9(n1950)	*ced-9(n1950)/ced-9(n1950)*	13.3±0.6	45

(b) *ced-9(n1950)* is a dominant gain-of-function mutation and shows a maternal effect

Maternal genotype	Zygotic genotype	Extra cells in anterior pharynx	No. of Animals
+/+	+/+	0.03±0.05	60
	Df/+	0.00	50
	ced-9(n1950)/+	5.3±0.8	25
ced-9(n1950)/+	+/+	0.2±0.22	25
	ced-9(n1950)/+	11.4±0.8	30
	ced-9(n1950)/ced-9(n1950)	13.7±0.5	30
ced-9(n1950)/ced-9(n1950)	*ced-9(n1950)*/+	11.8±0.6	30
	ced-9(n1950)/ced-9(n1950)	13.3±0.6	45

TABLE 1 (continued)

(c) *ced-9(n1950)* suppresses the accumulation of cell corpses

Genotype	Corpses Pharynx	n	Corpses Head	n
Wild-type (N2)	0	50	0.0±0.1	50
ced-1	0.8±0.2	100*	28	10†
ced-1; ced-3	0.02±0.04	50	0.3±0.1	50
ced-1; ced-4	0.02±0.04	50	0.7±0.2	50
ced-1; ced-9(n1950)	0	30	0.5±0.3	30
ced-5	3.6±0.6	25	16±5	10
ced-5; ced-3	0.1±0.1	40	0.5±0.2	40
ced-4; ced-5	0.2±0.2	40	1.0±0.3	40
ced-9(n1950); ced-5	0.1±0.1	100	0.8±0.4	25

Corpses			Extra Cells	
P9-P11	P12	Tail	P9-P11	n
0	0	0	0	30
3.5±0.3	1.7±0.3	1.7±0.3	0.4±0.3	30
0.03±0.07	0	0.3±0.2	3.9±0.1	30
0.03±0.07	0	0.3±0.2	4.0±0.1	30
0	0	0.3±0.2	4.0±0.1	30
3.0±0.5	2.2±0.3	4.6±0.8	0.2±0.2	21
0.05±0.10	0	0.4±0.4	3.9±0.1	20
0.05±0.10	0	1.6±0.5	3.9±0.2	20
0	0	1.1±0.3	3.8±0.1	30

TABLE 1 (continued)

(d) *ced-9(n1950)* prevents the deaths of the HSN neurons in egl-1 mutants

Genotype	HSNs missing (%)	No. of sides	Egg-laying defective (%)	n
Wild-type (N2)	1	250	0.4	704
egl-1	99	200	99	447
ced-3; egl-1	0	160	0.2	599
ced-4; egl-1	0	100	0	417
ced-9(n1950); egl-1	0	200	0	417

a. The genotypes of animals studied were as shown.

b. The complete genotypes are given in Example 1.

c. Extra cells, number of extra cells among the descendants of P9, P10, and P11. n, number of animals scored. *Data from Ellis et al., Genetics 129:79-94 (1991). †Data from Ellis and Horvitz, Cell 44:817-829 (1986).

d. HSN missing (%), percent of missing or grossly displaced HSN neurons. No. of sides, number of sides scored. n, number of animals scored.

Average numbers are shown with, if appropriate, their 95% confidence limits.

TABLE 2

Phenotypes of *ced-9(lf)* Mutants

Genotype	*ced-9(+)*	*ced-9(+)* / *Df*	*n1950 n2161*				*n1950 n2161* / *Df*	*n1950 n2161* / *n1950 n2077*
	20°C	20°C	15°C	20°C	23°C	25°C	20°C	20°C
(a) Sterility and maternal-effect lethality								
Eggs laid per animal	209±33	202±60	117±36	97±31	45±22	6.3±4.5	23±14	40±9
Hatching (%)	99±1	75±2	12±3	2.4±0.7	0.4±0.4	0	0	0
L1 arrest (%)	0	13±3	100	100	100	NA	NA	NA
	n=14	n=9	n=23	n=42	n=36	n=60	n=50	n=49
(b) Egg-laying defect								
Egg-laying defective (%)	0	0	64±16	76±13	94±7	98±3	96±4	96±6
	n=100	n=35	n=23	n=42	n=36	n=60	n=50	n=40
HSNs missing (%)	0	0	77	87	94	95	95	ND
	n=100	n=48	n=118	n=138	n=100	n=130	n=60	
(c) Absence of rays in male tails								
Rays per side	8.9±0.1	8.6±0.2	8.0±0.3	6.6±0.3	5.9±0.3	5.4±0.4	6.0±0.3	5.9±0.3
	n=68	n=34	n=40	n=40	n=58	n=34	n=62	n=38

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TABLE 2 (continued)

Phenotypes of *ced-9(lf)* Mutants

Genotype*	*n1653ts*	*n1653ts* / *Df*	*n1653ts* / *n1950 n2077*	*n1950 n2077*	*n1950 n2077* / *Df*
	25°C	25°C	25°C	20°C	20°C
(a) Sterility and maternal-effect lethality					
Eggs laid per animal	2.7±1.1	0	0.3±0.4	1.6±1.4	0.8±1.6
Hatching (%)	38±24	NA	0.7±1.4	0	0
L1 arrest (%)	40±36	NA	100	NA	NA
	n=26	n=15	n=30	n=20	n=24
(b) Egg-laying defect					
Egg-laying defective (%)	NA†	NA†	NA†	NA†	NA†
HSNs missing (%)	ND	ND	ND	99	100
				n=220	n=42
(c) Absence of rays in male tails					
Rays per side	8.6±0.2	7.6±0.3	8.1±0.3	4.6±0.3	4.9±0.6
	n=38	n=44	n=52	n=81	n=26

TABLE 2 (continued)

a. Hatching (%), percent of eggs laid that hatched within 48 hours of removal of the mother. L1 arrest (%), percent of hatched progeny that failed to develop past the first (L1) larval stage within 6 days of hatching. n, number of broods analysed.

b. For some genotypes (marked †) a significant fraction of the animals could not be scored accurately for egg-laying capability. n, number of animals scored. HSN missing (%), percent of missing or grossly displaced HSN neurons. n, number of sides scored.

c. Rays per side, number of rays present per side in the male tail. n, number of sides scored. Confidence limits (95%) were determined as in Table 1. NA, not applicable. *All strains were homozygous (*nDf40* strains were hemizygous) for the closely linked mutation *unc-69(3587)*.

TABLE 3

Mutations in *ced-3* and *ced-4* Suppress the Defects Resulting From the Loss of *ced-9* Function

	Sterility and Maternal-effect lethality			Egg-laying defect		Male tail	
Genotype*	Eggs laid per animal	Viable progeny	n	Egg-laying defective (%)	n	Rays per side	n
ced-9(+)	207±36	207±33	14	0	100	8.9±0.1	68
ced-9(n1950 n2077)	1.6±1.4	0	20	NA†	100	4.6±0.3	81
ced-4 ced-9(n1950 n2077)	200±19	160±20	12	1	84	9.0	42
ced-4	182±17	148±17	12	0	100	9.0±0.1	30
+ ced-9(n1950n 2077)							
ced-4 ced-9(n1950 n2077)	56±20	3.9±2.0	7	62	71	7.4±0.3	50
ced-9(n1950 n2077); ced-3	154±29	94±22	13	0	100	9.0	28
ced-3	178±51	146±46	6	0	66	8.9±0.1	50
ced-9(n1950 n2077); ced-3/+	132±62	17±6	10	18	40	8.7±0.1	50

Viable progeny, number of progeny that grew to the fourth larval (L4) stage within 10 days of hatching (this value includes a few animals that developed from eggs that hatched internally);

n, number of broods analysed.

Confidence limits (95%) were determined as in Table 1.

*All strains are homozygous for the closely linked mutation *unc-69(e587)*.

†Many animals could not be accurately scored for egg-laying.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT
(A) NAME: MASSACHUSETTS INSTITUTE OF TECHNOLOGY
(B) STREET: 77 Massachusetts Avenue
(C) CITY: Cambridge
(D) STATE OR PROVINCE: Massachusetts
(E) COUNTRY: U.S.A.
(F) POSTAL CODE: 02139

(ii) TITLE OF INVENTION: A Gene Which Prevents Programmed Cell Death

(iii) NUMBER OF SEQUENCES: 5

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: diskette

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6560 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATCGATAGTC GTCACCAAAT GGATTTTCCG ATTTCTCACT AGTCCATGGC TCACAATTTA 60

CAAAATCTCG AGAAAAGAAA GGATGCAAGG AGTATGAAGA GGTTCCGAAT CTAAATATTT 120

TAATTTAAAA AAATCAATTT CGAATTGAAA TTCAACTCCT ACTCGTTTTG AAAATGCCAA 180

TCCTTTAAGT AAACTTCTGG ATCGCCCATT TCTTCCAGAA ATTCCTTCAA AGTAGTGGTT 240

TTGTACTGAT TTCCTCCGCA AAGAATAGGA ACTTTCGAAT CTCCTGGAGC GAAACGGGAT 300

TTTSATAACA AAAAACTATC CAGACAAACC ATAGGACTTT TTCAAATATT CCTTATTTGG 360

CTGTCCATTT GGAAGCACCC AATCTTTAAC GCTGTCCAGC CAGAAGTGCT CCACTCGCCA 420

AGGATAAAAG GCTCATTTTT GAAGCCGAAT TTTACTAAAA TCTCTAGCCA TGGAGTCGAT 480

GGATCAGAAA TTCGAGGAAT TTTAGATTTC ATCTTGAAAT TTGCAATGGA AAAAATAATT 540

ATTCAAAGAA AATCACAGAA AATGCAACAA AAAAAACAAA AAAAGAACAA AAAACAAGTC 600

GAAAAGTGCG CCCGGGTCGT TTGCTGACGC ATCTCTTCAA ACGAGACGCG CTGCTGGCGC 660

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CCGCCCTGTT TCTTTTTTCA ATTTTGATAA GAAAATCAGC ATTGTTTCAG GATGATTAAC 780

ATTCCAACTG CGATTCTGTG CCGCTTGGGC GCCAGATCGT CGATTTCCCG CTCCTTTGGA 840

ACATCGATCG TCACCAAGGT GGGGATTTTT TGAATTTTTC CGTGAAAATT GTTGATTTTT 900

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CACCGAATCC GGCAAAACGG AATGGAATGG TTTGGAGCAC CGGGATTGCC GTGTGGAGTG 2040

CAACCGGAGC ACGAAATGAT GCGAGTTATG GGAACGATAT TCGAGAAGAA GCACGCGGAA 2100

AATTTTGAGA CCTTCTGTGA GCAGCTGCTC GCAGTGCCCA GAATCTCATT TTCACTGTAT 2160

CAGGATGTGG TTCGGACGGT TGGAAATGCA CAGACAGATC AATGTCCAAT GTCTTATGGA 2220

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TATCCCCCTT TCCCTAGTAC CCTTGACTTC CCGGGGTGTT GGTAAGCCGA TAATTACAGG 3000

GTTCGGTAGC CTCTTGGGGG GACAGCTGGA AACATATTCA AGTATATTAC TGTTTATGAT 3060

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GCAAAATATC CAGTAGAGAA AACTACAGTA ATTCTTTAAA TTTTTAAAAT TTTTACAATT 3180

AAAGAAAATA ACCACTAATC AAAAGAAATT AATTTCAAAA ATCGAGCCCG TAAATCGACT 3240

ACAGTAGGCA TTTAAAGAAT TACTGTAGTT TTCGCTACGA GATATTTCCG CCTCAAATAT 3300

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GCGACCACTA TGACTGTGAT CGTGGCAAGA CCCACTCAGA AAACTACGTG TTCCTTTAAA 6360

CAAATACATT TTTAAGTATT GTAGGTATAA AAATTGTTGG CTAGCAGTCT AGGCTGCCTT 6420

TTTCAGTCGA CAAACTTCTA ATTTAATCGG CGGGTCTTCA AAAAGTCGTT TCTTTGAAAA 6480

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TATAAAGCTT TATATATTTA TATATTAAAA ATTTTGATTA CATGATATCA AAAGCGACTA    6540

GTTTGTATAA AAATTATCAA                                                6560
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1315 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 7..846
(D) OTHER INFORMATION: /product= "Ced-9"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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       Met Thr Arg Cys Thr Ala Asp Asn Ser Leu Thr Asn Pro Ala
         1               5                  10

TAT CGG CGA CGA ACG ATG GCG ACT GGC GAG ATG AAG GAG TTT CTG GGG       96
Tyr Arg Arg Arg Thr Met Ala Thr Gly Glu Met Lys Glu Phe Leu Gly
 15                  20                  25                  30

ATA AAA GGC ACA GAG CCC ACC GAT TTT GGA ATC AAT AGT GAT GCT CAG      144
Ile Lys Gly Thr Glu Pro Thr Asp Phe Gly Ile Asn Ser Asp Ala Gln
                35                  40                  45

GAC TTG CCA TCA CCG AGT AGG CAG GCT TCG ACG CGA AGA ATG TCC ATC      192
Asp Leu Pro Ser Pro Ser Arg Gln Ala Ser Thr Arg Arg Met Ser Ile
            50                  55                  60

GGA GAG TCA ATT GAT GGA AAA ATC AAT GAT TGG GAA GAG CCA AGG CTT      240
Gly Glu Ser Ile Asp Gly Lys Ile Asn Asp Trp Glu Glu Pro Arg Leu
        65                  70                  75

GAT ATC GAG GGA TTT GTG GTC GAC TAT TTC ACG CAC CGA ATC CGG CAA      288
Asp Ile Glu Gly Phe Val Val Asp Tyr Phe Thr His Arg Ile Arg Gln
    80                  85                  90

AAC GGA ATG GAA TGG TTT GGA GCA CCG GGA TTG CCG TGT GGA GTG CAA      336
Asn Gly Met Glu Trp Phe Gly Ala Pro Gly Leu Pro Cys Gly Val Gln
95                  100                 105                 110

CCG GAG CAC GAA ATG ATG CGA GTT ATG GGA ACG ATA TTC GAG AAG AAG      384
Pro Glu His Glu Met Met Arg Val Met Gly Thr Ile Phe Glu Lys Lys
                115                 120                 125

CAC GCG GAA AAT TTT GAG ACC TTC TGT GAG CAG CTG CTC GCA GTG CCC      432
His Ala Glu Asn Phe Glu Thr Phe Cys Glu Gln Leu Leu Ala Val Pro
            130                 135                 140

AGA ATC TCA TTT TCA CTG TAT CAG GAT GTG GTT CGG ACG GTT GGA AAT      480
Arg Ile Ser Phe Ser Leu Tyr Gln Asp Val Val Arg Thr Val Gly Asn
        145                 150                 155
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GCA CAG ACA GAT CAA TGT CCA ATG TCT TAT GGA CGT TTG ATA GGT CTA        528
Ala Gln Thr Asp Gln Cys Pro Met Ser Tyr Gly Arg Leu Ile Gly Leu
    160                 165                 170

ATC TCG TTC GGC GGT TTC GTA GCT GCA AAA ATG ATG GAA TCC GTG GAA        576
Ile Ser Phe Gly Gly Phe Val Ala Ala Lys Met Met Glu Ser Val Glu
175                 180                 185                 190

CTG CAG GGA CAA GTG CGA AAC CTC TTC GTT TAC ACA TCG CTG TTC ATC        624
Leu Gln Gly Gln Val Arg Asn Leu Phe Val Tyr Thr Ser Leu Phe Ile
                195                 200                 205

AAA ACG CGG ATC CGC AAC AAC TGG AAG GAA CAC AAT CGG AGC TGG GAC        672
Lys Thr Arg Ile Arg Asn Asn Trp Lys Glu His Asn Arg Ser Trp Asp
            210                 215                 220

GAC TTC ATG ACA CTC GGA AAA CAA ATG AAA GAG GAC TAC GAA CGA GCA        720
Asp Phe Met Thr Leu Gly Lys Gln Met Lys Glu Asp Tyr Glu Arg Ala
        225                 230                 235

GAA GCT GAA AAA GTG GGA CGC CGG AAG CAG AAC AGA CGG TGG TCG ATG        768
Glu Ala Glu Lys Val Gly Arg Arg Lys Gln Asn Arg Arg Trp Ser Met
    240                 245                 250

ATT GGC GCT GGA GTA ACA GCT GGA GCC ATT GGA ATC GTT GGA GTC GTC        816
Ile Gly Ala Gly Val Thr Ala Gly Ala Ile Gly Ile Val Gly Val Val
255                 260                 265                 270

GTG TGT GGG CGG ATG ATG TTC AGC TTG AAG TAACGTATTC AATTTGTGTA          866
Val Cys Gly Arg Met Met Phe Ser Leu Lys
                275                 280

AATAATTAAT TTATGTACAA CTCCTTACAT TTGAATCTCA TTTTKGCTCA CTGATTCTCT      926

CATCCTTTGA ACTGGAAGAA GTGGGAAAGC TAGGCCACAA ATTACGGCTC TCTGTGTCGA      986

TTTACGATTT TACTGCAATT TTTTCCGATT GCCTTTTTTT TTGGCCAAAC CCTACTTCCG     1046

CGTAATATCA ACTTTTCCGT GTTCTGTACA TTTCGTCAAA AACCCTGAAA CCCTAACTTT     1106

TCTCGCCGTG GCCTAGCCTC CCGCTTCTCT TCCACATTTC CAAAGTACCC CTGTATCTCA     1166

ATAATTCATC TTCACTTTAA CTGTCTCTTT TCGTGTGGCC TCTTCCAACT CCCCCCAAAT     1226

TCCTGTACGC GTACGCGACT TTGTATTTAT TTTTTTCAAA TTGTTTTCTC TCTACAACAA     1286

CAAAAAAAAC GGTTCAAAAA AAAAAAAAA                                       1315
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 280 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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Met Thr Arg Cys Thr Ala Asp Asn Ser Leu Thr Asn Pro Ala Tyr Arg
 1               5                  10                  15
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Arg Arg Thr Met Ala Thr Gly Glu Met Lys Glu Phe Leu Gly Ile Lys
                20                  25                  30

Gly Thr Glu Pro Thr Asp Phe Gly Ile Asn Ser Asp Ala Gln Asp Leu
            35                  40                  45

Pro Ser Pro Ser Arg Gln Ala Ser Thr Arg Arg Met Ser Ile Gly Glu
        50                  55                  60

Ser Ile Asp Gly Lys Ile Asn Asp Trp Glu Glu Pro Arg Leu Asp Ile
65                  70                  75                  80

Glu Gly Phe Val Val Asp Tyr Phe Thr His Arg Ile Arg Gln Asn Gly
                85                  90                  95

Met Glu Trp Phe Gly Ala Pro Gly Leu Pro Cys Gly Val Gln Pro Glu
            100                 105                 110

His Glu Met Met Arg Val Met Gly Thr Ile Phe Glu Lys Lys His Ala
        115                 120                 125

Glu Asn Phe Glu Thr Phe Cys Glu Gln Leu Leu Ala Val Pro Arg Ile
    130                 135                 140

Ser Phe Ser Leu Tyr Gln Asp Val Val Arg Thr Val Gly Asn Ala Gln
145                 150                 155                 160

Thr Asp Gln Cys Pro Met Ser Tyr Gly Arg Leu Ile Gly Leu Ile Ser
                165                 170                 175

Phe Gly Gly Phe Val Ala Ala Lys Met Met Glu Ser Val Glu Leu Gln
            180                 185                 190

Gly Gln Val Arg Asn Leu Phe Val Tyr Thr Ser Leu Phe Ile Lys Thr
        195                 200                 205

Arg Ile Arg Asn Asn Trp Lys Glu His Asn Arg Ser Trp Asp Asp Phe
    210                 215                 220

Met Thr Leu Gly Lys Gln Met Lys Glu Asp Tyr Glu Arg Ala Glu Ala
225                 230                 235                 240

Glu Lys Val Gly Arg Arg Lys Gln Asn Arg Arg Trp Ser Met Ile Gly
                245                 250                 255

Ala Gly Val Thr Ala Gly Ala Ile Gly Ile Val Gly Val Val Val Cys
            260                 265                 270

Gly Arg Met Met Phe Ser Leu Lys
        275                 280
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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5086 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1459..2178
(D) OTHER INFORMATION: /product= "Bcl-2"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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TCCCTGCCGG CGGCCGTCAG CGCTCGGAGC GAACTGCGCG ACGGGAGGTC CGGGAGGCGA     180

CCGTAGTCGC GCCGCCGCGC AGGACCAGGA GGAGGAGAAA GGGTGCGCAG CCCGGAGGCG     240

GGGTGCGCCG GTGGGGTGCA GCGGAAGAGG GGGTCCAGGG GGGAGAACTT CGTAGCAGTC     300

ATCCTTTTTA GGAAAAGAGG GAAAAAATAA AACCCTCCCC CACCACCTCC TTCTCCCCAC     360

CCCTCGCCGC ACCACACACA GCGCGGGCTT CTAGCGCTCG GCACCGGCGG GCCAGGCGCG     420

TCCTGCCTTC ATTTATCCAG CAGCTTTTCG GAAAATGCAT TTGCTGTTCG GAGTTTAATC     480

AGAAGACGAT TCCTGCCTCC GTCCCCGGCT CCTTCATCGT CCCATCTCCC CTGTCTCTCT     540

CCTGGGGAGG CGTGAAGCGG TCCCGTGGAT AGAGATTCAT GCCTGTGTCC GCGCGTGTGT     600

GCGCGCGTAT AAATTGCCGA GAAGGGGAAA ACATCACAGG ACTTCTGCGA ATACCGGACT     660

GAAAATTGTA ATTCATCTGC CGCCGCCGCT GCCAAAAAAA AACTCGAGCT CTTGAGATCT     720

CCGGTTGGGA TTCCTGCGGA TTGACATTTC TGTGAAGCAG AAGTCTGGGA ATCGATCTGG     780

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TCGCACAGGA AATTGGTTTA ATGTAACTTT CAATGGAAAC CTTTGAGATT TTTTACTTAA    1020

AGTGCATTCG AGTAAATTTA ATTTCCAGGC AGCTTAATAC ATTGTTTTTA GCCGTGTTAC    1080

TTGTAGTGTG TATGCCCTGC TTTCACTCAG TGTGTACAGG GAAACGCACC TGATTTTTTA    1140

CTTATTAGTT TGTTTTTTCT TTAACCTTTC AGCATCACAG AGGAAGTAGA CTGATATTAA    1200

CAATACTTAC TAATAATAAC GTGCCTCATG AAATAAAGAT CCGAAAGGAA TTGGAATAAA    1260

AATTTCCTGC GTCTCATGCC AAGAGGGAAA CACCAGAATC AAGTGTTCCG CGTGATTGAA    1320

GACACCCCCT CGTCCAAGAA TGCAAAGCAC ATCCAATAAA ATAGCTGGAT TATAACTCCT    1380

CTTCTTTCTC TGGGGGCCGT GGGGTGGGAG CTGGGGCGAG AGGTGCCGTT GGCCCCCGTT    1440

GCTTTTCCTC TGGGAAGG ATG GCG CAC GCT GGG AGA ACG GGG TAC GAC AAC       1491
                      Met Ala His Ala Gly Arg Thr Gly Tyr Asp Asn
                        1               5                  10

CGG GAG ATA GTG ATG AAG TAC ATC CAT TAT AAG CTG TCG CAG AGG GGC      1539
Arg Glu Ile Val Met Lys Tyr Ile His Tyr Lys Leu Ser Gln Arg Gly
            15                  20                  25
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TAC GAG TGG GAT GCG GGA GAT GTG GGC GCC GCG CCC CCG GGG GCC GCC      1587
Tyr Glu Trp Asp Ala Gly Asp Val Gly Ala Ala Pro Pro Gly Ala Ala
         30                  35                  40

CCC GCA CCG GGC ATC TTC TCC TCC CAG CCC GGG CAC ACG CCC CAT CCA      1635
Pro Ala Pro Gly Ile Phe Ser Ser Gln Pro Gly His Thr Pro His Pro
     45                  50                  55

GCC GCA TCC CGC GAC CCG GTC GCC AGG ACC TCG CCG CTG CAG ACC CCG      1683
Ala Ala Ser Arg Asp Pro Val Ala Arg Thr Ser Pro Leu Gln Thr Pro
 60                  65                  70                  75

GCT GCC CCC GGC GCC GCC GCG GGG CCT GCG CTC AGC CCG GTG CCA CCT      1731
Ala Ala Pro Gly Ala Ala Ala Gly Pro Ala Leu Ser Pro Val Pro Pro
                 80                  85                  90

GTG GTC CAC CTG GCC CTC CGC CAA GCC GGC GAC GAC TTC TCC CGC CGC      1779
Val Val His Leu Ala Leu Arg Gln Ala Gly Asp Asp Phe Ser Arg Arg
             95                 100                 105

TAC CGC GGC GAC TTC GCC GAG ATG TCC AGC CAG CTG CAC CTG ACG CCC      1827
Tyr Arg Gly Asp Phe Ala Glu Met Ser Ser Gln Leu His Leu Thr Pro
        110                 115                 120

TTC ACC GCG CGG GGA CGC TTT GCC ACG GTG GTG GAG GAG CTC TTC AGG      1875
Phe Thr Ala Arg Gly Arg Phe Ala Thr Val Val Glu Glu Leu Phe Arg
    125                 130                 135

GAC GGG GTG AAC TGG GGG AGG ATT GTG GCC TTC TTT GAG TTC GGT GGG      1923
Asp Gly Val Asn Trp Gly Arg Ile Val Ala Phe Phe Glu Phe Gly Gly
140                 145                 150                 155

GTC ATG TGT GTG GAG AGC GTC AAC CGG GAG ATG TCG CCC CTG GTG GAC      1971
Val Met Cys Val Glu Ser Val Asn Arg Glu Met Ser Pro Leu Val Asp
                160                 165                 170

AAC ATC GCC CTG TGG ATG ACT GAG TAC CTG AAC CGG CAC CTG CAC ACC      2019
Asn Ile Ala Leu Trp Met Thr Glu Tyr Leu Asn Arg His Leu His Thr
            175                 180                 185

TGG ATC CAG GAT AAC GGA GGC TGG GAT GCC TTT GTG GAA CTG TAC GGC      2067
Trp Ile Gln Asp Asn Gly Gly Trp Asp Ala Phe Val Glu Leu Tyr Gly
        190                 195                 200

CCC AGC ATG CGG CCT CTG TTT GAT TTC TCC TGG CTG TCT CTG AAG ACT      2115
Pro Ser Met Arg Pro Leu Phe Asp Phe Ser Trp Leu Ser Leu Lys Thr
    205                 210                 215

CTG CTC AGT TTG GCC CTG GTG GGA GCT TGC ATC ACC CTG GGT GCC TAT      2163
Leu Leu Ser Leu Ala Leu Val Gly Ala Cys Ile Thr Leu Gly Ala Tyr
220                 225                 230                 235

CTG AGC CAC AAG TGAAGTCAAC ATGCCTGCCC CAAACAAATA TGCAAAAGGT          2215
Leu Ser His Lys
                240

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GCTGTTTAAG AAAAAATAAC ACACATATAA ACATCACACA CACAGACAGA CACACACACA    2335

CACAACAATT AACAGTCTTC AGGCAAAACG TCGAATCAGC TATTTACTGC CAAAGGGAAA    2395
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TGGATGTTCT GTGCCTGTAA ACATAGATTC GCTTTCCATG TTGTTGGCCG GATCACCATC 2575

TGAAGAGCAG ACGGATGGAA AAAGGACCTG ATCATTGGGG AAGCTGGCTT TCTGGCTGCT 2635

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TCATAGGAAA GTATTTTTTT AAGCTACCAA TTGTGCCGAG AAAAGCATTT TAGCAATTTA 2935

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GAGGCCTGGT CCTGGAACTG AGCCGGGCCC TCACTGGCCT CCTCCAGGGA TGATCAACAG 3235

GGTAGTGTGG TCTCCGAATG TCTGGAAGCT GATGGATGGA GCTCAGAATT CCACTGTCAA 3295

GAAAGAGCAG TAGAGGGGTG TGGCTGGGCC TGTCACCCTG GGGCCCTCCA GGTAGGCCCG 3355

TTTTCACGTG GAGCATAGGA GCCACGACCC TTCTTAAGAC ATGTATCACT GTAGAGGGAA 3415

GGAACAGAGG CCCTGGGCCT TCCTATCAGA AGGACATGGT GAAGGCTGGG AACGTGAGGA 3475

GAGGCAATGG CCACGGCCCA TTTTGGCTGT AGCACATGGC ACGTTGGCTG TGTGGCCTTG 3535

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AGAAGCATTG AAGTGAGGTG TCATGGATTA ATTGACCCCT GTCTATGGAA TTACATGTAA 3655

AACATTATCT TGTCACTGTA GTTTGGTTTT ATTTGAAAAC CTGACAAAAA AAAAGTTCCA 3715

GGTGTGGAAT ATGGGGGTTA TCTGTACATC CTGGGGCATT AAAAAAAAAT CAATGGTGGG 3775

GAACTATAAA GAAGTAACAA AAGAAGTGAC ATCTTCAGCA AATAAACTAG GAAATTTTTT 3835

TTTCTTCCAG TTTAGAATCA GCCTTGAAAC ATTGATGGAA TAACTCTGTG GCATTATTGC 3895

ATTATATACC ATTTATCTGT ATTAACTTTG GAATGTACTC TGTTCAATGT TTAATGCTGT 3955

GGTTGATATT TCGAAAGCTG CTTTAAAAAA ATACATGCAT CTCAGCGTTT TTTTGTTTTT 4015

AATTGTATTT AGTTATGGCC TATACACTAT TTGTGAGCAA AGGTGATCGT TTTCTGTTTG 4075

AGATTTTTAT CTCTTGATTC TTCAAAAGCA TTCTGAGAAG GTGAGATAAG CCCTGAGTCT 4135

CAGCTACCTA AGAAAAACCT GGATGTCACT GGCCACTGAG GAGCTTTGTT TCAACCAAGT 4195

CATGTGCATT TCCACGTCAA CAGAATTGTT TATTGTGACA GTTATATCTG TTGTCCCTTT 4255

GACCTTGTTT CTTGAAGGTT TCCTCGTCCC TGGGCAATTC CGCATTTAAT TCATGGTATT 4315

CAGGATTACA TGCATGTTTG GTTAAACCCA TGAGATTCAT TCAGTTAAAA ATCCAGATGG 4375

CGAATGACCA GCAGATTCAA ATCTATGGTG GTTTGACCTT TAGAGAGTTG CTTTACGTGG 4435

CCTGTTTCAA CACAGACCCA CCCAGAGCCC TCCTGCCCTC CTTCCGCGGG GGCTTTCTCA 4495

TGGCTGTCCT TCAGGGTCTT CCTGAAATGC AGTGGTCGTT ACGCTCCACC AAGAAAGCAG 4555

GAAACCTGTG GTATGAAGCC AGACCTCCCC GGCGGGCCTC AGGGAACAGA ATGATCAGAC 4615

CTTTGAATGA TTCTAATTTT TAAGCAAAAT ATTATTTTAT GAAAGGTTTA CATTGTCAAA 4675

GTGATGAATA TGGAATATCC AATCCTGTGC TGCTATCCTG CCAAAATCAT TTTAATGGAG 4735

TCAGTTTGCA GTATGCTCCA CGTGGTAAGA TCCTCCAAGC TGCTTTAGAA GTAACAATGA 4795

AGAACGTGGA CGTTTTTAAT ATAAAGCCTG TTTTGTCTTT TGTTGTTGTT CAAACGGGAT 4855

TCACAGAGTA TTTGAAAAAT GTATATATAT TAAGAGGTCA CGGGGGCTAA TTGCTAGCTG 4915

GCTGCCTTTT GCTGTGGGGT TTTGTTACCT GGTTTTAATA ACAGTAAATG TGCCCAGCCT 4975

CTTGGCCCCA GAACTGTACA GTATTGTGGC TGCACTTGCT CTAAGAGTAG TTGATGTTGC 5035

ATTTTCCTTA TTGTTAAAAA CATGTTAGAA GCAATGAATG TATATAAAAG C 5086

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 239 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Ala His Ala Gly Arg Thr Gly Tyr Asp Asn Arg Glu Ile Val Met
1 5 10 15

Lys Tyr Ile His Tyr Lys Leu Ser Gln Arg Gly Tyr Glu Trp Asp Ala
20 25 30

Gly Asp Val Gly Ala Ala Pro Pro Gly Ala Ala Pro Ala Pro Gly Ile
35 40 45

Phe Ser Ser Gln Pro Gly His Thr Pro His Pro Ala Ala Ser Arg Asp
50 55 60

Pro Val Ala Arg Thr Ser Pro Leu Gln Thr Pro Ala Ala Pro Gly Ala
65 70 75 80

Ala Ala Gly Pro Ala Leu Ser Pro Val Pro Pro Val Val His Leu Ala
85 90 95

Leu Arg Gln Ala Gly Asp Asp Phe Ser Arg Arg Tyr Arg Gly Asp Phe
100 105 110

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Ala Glu Met Ser Ser Gln Leu His Leu Thr Pro Phe Thr Ala Arg Gly
        115                 120                 125

Arg Phe Ala Thr Val Val Glu Glu Leu Phe Arg Asp Gly Val Asn Trp
    130                 135                 140

Gly Arg Ile Val Ala Phe Phe Glu Phe Gly Gly Val Met Cys Val Glu
145                 150                 155                 160

Ser Val Asn Arg Glu Met Ser Pro Leu Val Asp Asn Ile Ala Leu Trp
                165                 170                 175

Met Thr Glu Tyr Leu Asn Arg His Leu His Thr Trp Ile Gln Asp Asn
            180                 185                 190

Gly Gly Trp Asp Ala Phe Val Glu Leu Tyr Gly Pro Ser Met Arg Pro
        195                 200                 205

Leu Phe Asp Phe Ser Trp Leu Ser Leu Lys Thr Leu Leu Ser Leu Ala
    210                 215                 220

Leu Val Gly Ala Cys Ile Thr Leu Gly Ala Tyr Leu Ser His Lys
225                 230                 235
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CLAIMS

1. Substantially pure nucleic acid which is a cell death-protective gene.

2. The nucleic acid of Claim 1, wherein the cell death-protective gene is the *C. elegans ced-9* gene.

3. Substantially pure nucleic acid consisting essentially of all or a portion of the nucleotide sequence selected from the group consisting of:
   a) the nucleotide sequence shown in Figure 2 (SEQ ID NO:1); and
   b) the nucleotide sequence shown in Figure 3 (SEQ ID NO:2).

4. Substantially pure nucleic acid encoding the amino acid sequence shown in Figure 4 (SEQ ID NO:3).

5. Isolated protein encoded by the *C. elegans ced-9* gene.

6. The protein of Claim 5 consisting essentially of the amino acid sequence shown in Figure 4 (SEQ ID NO:3).

7. An antibody specifically reactive with the protein of Claim 5.

8. Substantially pure nucleic acid which is a mutated cell death-protective gene, wherein the mutation constitutively activates the gene.

9. The nucleic acid of Claim 8, wherein the cell death-protective gene is *C. elegans ced-9*.

10. Isolated protein encoded by the nucleic acid of Claim 9.

11. The nucleic acid of Claim 9, wherein the mutation is n1950.

12. The nucleic acid of Claim 11, wherein the mutation is associated with a glycine to glutamic acid change at codon 169.

13. The nucleic acid of Claim 8, wherein the cell death-protective gene is human bcl-2.

14. Isolated protein encoded by the nucleic acid of Claim 13.

15. The nucleic acid of Claim 13, wherein the mutation is associated with a glycine to glutamic acid change at codon 145.

16. Substantially pure nucleic acid which is a mutated cell death-protective gene, wherein the mutation inactivates the gene.

17. Isolated protein encoded by the nucleic acid of Claim 16.

18. The nucleic acid of Claim 16, wherein the cell death-protective gene is C. elegans ced-9.

19. The nucleic acid of Claim 18, wherein the mutation is selected from the group consisting of:

a) n2077;

b) n2161; and

c) n1653ts.

20. The nucleic acid of Claim 18, wherein the mutation is associated with a change selected from the group consisting of:

a) tyrosine to asparagine at codon 149; and

b) glutamine to termination at codon 160.

21. The nucleic acid of Claim 16, wherein the cell death-protective gene is human bcl-2.

22. Use of an agent which alters the activity of a cell death-protective gene (e.g. ced-9 or bcl-2) or a novel human cell death-protective gene structurally similar to ced-9 or bcl-2 in a cell (e.g. a nematode cell or a human cell), for the manufacture of a medicament for treating a condition in which the occurrence of cell death is at an abnormal rate.

23. Use according to Claim 22, wherein the cell death-protective gene is activated by a mutation, and protects against cell death.

24. Use according to Claim 23, wherein the cell death-protective gene is ced-9 and the mutation is n1950.

25. Use according to Claim 24, wherein the mutation is associated with a change from glycine to glutamic acid at codon 169.

26. Use according to Claim 23, wherein the cell death-protective gene is bcl-2, and the mutation is associated with a change from glycine to glutamic acid at codon 145.

27. Use accoarding to Claim 22, wherein the cell death-protective gene is inactivated by a mutation, and does not provide protection against programmed cell death.

28. Use according to Claim 27, wherein the cell death-protective gene is C. elegans ced-9 and the mutation is selected from the group consisting of:
a) n2077;
b) n2161; and
c) n1653ts.

29. Use according to Claim 22, wherein the activity of the cell death-protective gene is increased, and the agent is selected from the group consisting of:
a) DNA comprising the cell death-protective gene;
b) RNA encoded by the cell death-protective gene;
c) protein encoded by the cell death-protective gene, or active portion thereof;
d) an agent which mimics the activity of the cell death-protective gene;
e) an activator of the cell death protective gene; and
f) an agonist of the cell death-protective gene.

30. Use according to Claim 22, wherein the activity of the cell death-protective gene is decreased, and the agent is selected from the group consisting of:
a) single stranded nucleic acid which is complementary to the mRNA of the cell death-protective gene;
b) DNA which directs the expression of a);

c) a mutated cell death-protective gene which does not protect from cell death and which antagonizes the activity of the cell death-protective gene;

d) an inactivator of the cell death-protective gene; and

e) an antagonist of the cell death-protective gene.

31. Use according to Claim 22, wherein the medicament is for treating in a population of cells a condition characterized by increased cell deaths, the agent is selected from the group consisting of:

a) DNA comprising the cell death-protective gene;

b) RNA encoded by the cell death-protective gene;

c) protein encoded by the cell death-protective gene, or active portion thereof;

d) an agent which mimics the activity of the cell death-protective gene;

e) an activator of the cell death protective gene; and

f) an agonist of the cell death-protective gene, under conditions appropriate for activity of the agent.

32. Use according to Claim 31, wherein the medicament is for treating any of:

a) myocardial infarction;

b) stroke;

c) traumatic brain injury;

d) neurodegenerative disease;

e) muscular degenerative disease;

f) aging;

g) hypoxia;

h) ischemia;

i) toxemia;
j) infection, for example a virus infection e.g. human immunodeficiency virus infection; and
k) hair loss.

33. Use of any of:
a) single stranded nucleic acid which is complementary to the mRNA of the cell death-protective gene;
b) DNA which directs the expression of a);
c) a mutated cell death-protective gene which does not protect from cell death and which antagonizes the activity of the cell death-protective gene;
d) an inactivator of the cell death-protective gene; and
e) an antagonist of the cell death-protective gene,
for the manufacture of a medicament for reducing a population of cells, comprising decreasing the activity of a cell death-protective gene (e.g. ced-9 or bcl-2 or a novel human cell death-protective gene structurally similar to ced-9 or bcl-2) in the treatment of for example any of:
a) neoplastic growth;
b) cancerous tissue;
c) infected cells; and
d) autoreactive immune cells.

34. Use according to Claim 22, wherein the medicament is for treating a parasitic infection (e.g. a nematode infection) of a host animal, and the agent is adapted to decrease the activity of a cell death-protective gene specific to the parasite without harming the host animal.

35. Use according to Claim 34, wherein the agent is selected from:

a) single stranded nucleid acid having all or a portion of the antisense sequence of the cell death-protective gene which is complementary to the mRNA of the gene; and

b) DNA which encodes a).

36. A method for altering cell death in a cell, comprising altering in the cell the activity of a cell death-protective gene.

37. The method of Claim 36, wherein the cell death-protective gene is ced-9 or bcl-2.

38. The method of Claim 36, wherein the cell is a nematode cell or a human cell.

39. The method of Claim 36, wherein cell death is prevented and the cell death-protective gene is activated and protects against cell death.

40. The method of Claim 36, wherein the cell death-protective gene is activated by a mutation.

41. The method of Claim 40, wherein the cell death-protective gene is ced-9 and the mutation is n1950.

42. The method of Claim 41, wherein the mutation is associated with a change from glycine to glutamic acid at codon 169.

43. The method of Claim 40, wherein the cell death-protective gene is bcl-2.

44. The method of Claim 43, wherein the mutation is associated with a change from glycine to glutamic acid at codon 145.

45. The method of Claim 36, wherein cell death is caused and the cell death-protective gene is inactivated and does not provide protection against programmed cell death.

46. The method of Claim 45, wherein the cell death-protective gene is inactivated by a mutation.

47. The method of Claim 46, wherein the cell death-protective gene is C. elegans ced-9 and the mutation is selected from the group consisting of:
a) n2077;
b) n2161; and
c) n1653ts.

48. The method of Claim 46, comprising exposing the cell to an agent which alters the activity of a cell death-protective gene in the cell under conditions appropriate for activity of the agent.

49. The method of Claim 48, wherein the cell death-protective gene is ced-9 or bcl-2.

50. The method of Claim 48, wherein the activity of the cell death-protective gene is increased, said method comprising exposing the cell to an agent selected from the group consisting of:
a) DNA comprising the cell death-protective gene;
b) RNA encoded by the cell death-protective gene;
c) protein encoded by the cell death-protective gene, or active portion thereof;

d) an agent which mimics the activity of the cell death-protective gene;

e) an activator of the cell death protective gene; and

f) an agonist of the cell death-protective gene.

51. The method of Claim 48, wherein the activity of the cell death-protective gene is decreased, said method comprising exposing the cell to an agent selected from the group consisting of:

a) single stranded nucleic acid which is complementary to the mRNA of the cell death-protective gene;

b) DNA which directs the expression of a);

c) a mutated cell death-protective gene which does not protect from cell death and which antagonizes the activity of the cell death-protective gene;

d) an inactivator of the cell death-protective gene; and

e) an antagonist of the cell death-protective gene.

52. A method for treating in a population of cells a condition characterized by increased cell deaths, comprising increasing in the population of cells the activity of a cell death-protective gene which functions in the cells.

53. The method of Claim 52, wherein the tissue is exposed to an agent selected from the group consisting of:

a) DNA comprising the cell death-protective gene;

b) RNA encoded by the cell death-protective gene;

c) protein encoded by the cell death-protective gene, or active portion thereof;

d) an agent which mimics the activity of the cell death-protective gene;

e) an activator of the cell death protective gene; and

f) an agonist of the cell death-protective gene,

under conditions appropriate for activity of the agent.

54. The method of Claim 52, wherein the cell death-protective gene is selected from the group consisting of:

a) bcl-2;

b) a novel human cell death-protective gene which is structurally similar to bcl-2; and

c) a novel human cell death-protective gene which is structurally similar to ced-9.

55. The method of Claim 53, wherein the condition is selected from the group consisting of:

a) myocardial infarction;

b) stroke;

c) traumatic brain injury;

d) neurodegenerative disease;

e) muscular degenerative disease;

f) aging;

g) hypoxia;

h) ischemia;

i) toxemia;

j) infection; and

k) hair loss.

56. The method of Claim 55, wherein the condition is a viral infection.

57. The method of Claim 56, wherein the viral infection is with human immunodeficiency virus.

58. A method for reducing a population of cells, comprising decreasing the activity of a cell death-protective gene which functions in the cells.

59. The method of Claim 58, wherein the cells are exposed to an agent selected from the group consisting of:

a) single stranded nucleic acid which is complementary to the mRNA of the cell death-protective gene;

b) DNA which directs the expression of a);

c) a mutated cell death-protective gene which does not protect from cell death and which antagonizes the activity of the cell death-protective gene;

d) an inactivator of the cell death-protective gene; and

e) an antagonist of the cell death-protective gene,

under conditions appropriate for the activity of the agent.

60. The method of Claim 58, wherein the population of cells is selected from the group consisting of:

a) neoplastic growth;

b) cancerous tissue;

c) infected cells; and

d) autoreactive immune cells.

61. The method of Claim 58, wherein the cell death-protective gene is selected from the group consisting of:

a) bcl-2;
b) a novel human cell death-protective gene which is structurally similar to bcl-2; and
c) a novel human cell death-protective gene which is structurally similar to ced-9.

62. A method for treating a parasitic infection of a host animal, comprising the steps of administering an agent which decreases the activity of a cell death-protective gene specific to the parasite and does not harm the host animal.

63. The method of Claim 62, wherein the agent is selected from:
a) single stranded nucleid acid having all or a portion of the antisense sequence of the cell death-protective gene which is complementary to the mRNA of the gene; and
b) DNA which encodes a).

64. The method of Claim 62, wherein the parasite is a nematode.

65. A method of pest control, comprising decreasing the activity of a cell death-protective gene in the pest.

66. The method of Claim 65, comprising exposing the pest to an agent selected from the group consisting of:
a) single stranded nucleic acid having all or a portion of the antisense sequence of the cell death-protective gene which is complementary to the mRNA of the gene;
b) DNA which directs the expression of a);

c) a mutated cell death-protective gene which does not protect from cell death and which antagonizes the activity of the cell death-protective gene;

d) an inactivator of the cell death-protective gene; and

e) an antagonist of the cell death-protective gene,

under conditions appropriate for the activity of the agent.

67. A method of biological containment of a recombinant organism, comprising introducing in the organism nucleic acid which is able to direct the expression of an agent which inactivates a cell death-protective gene in the organism under predetermined conditions.

68. The method of Claim 67, wherein the agent is selected from the group consisting of:

a) single stranded nucleic acid which has all or a portion of the antisense sequence of the cell death-protective gene which is complementary to the mRNA of the gene;

b) DNA which directs the expression of a);

c) a mutated cell death-protective gene which does not protect from cell death and which antagonizes the activity of the cell death-protective gene; and

d) an inactivator of the cell death-protective gene.

69. The method of Claim 68, wherein the agent kills the recombinant organism upon completion of a desired task by the organism.

70. A method for identifying a novel cell death-protective gene, comprising:

a) obtaining a structurally similar gene by:

1) identifying a gene which is structurally similar to a cell death-protective gene; or

2) identifying a gene whose gene product is similar to the gene product of a cell death-protective gene; and

b) determining that the structurally similar gene protects cells in which it functions from cell death,

thereby identifying a novel cell death-protective gene.

71. The method of Claim 70, wherein the cell death-protective gene is ced-9 or bcl-2.

72. Substantially pure nucleic acid which is a gene identified by the method of Claim 71.

73. The method of Claim 70, wherein step a) comprises the steps of:

a) combining DNA with a nucleic acid probe comprising the cell death-protective gene, or a portion able to specifically hybridize to the cell death-protective gene, under conditions suitable for specific hybridization of the nucleic acid probe to complementary sequences; and

b) detecting specific hybridization of the nucleic acid probe to the DNA, wherein specific hybridization indicates that a structurally similar gene, or portion, is present in the DNA,

thereby identifying a gene which is structurally similar to a cell death-protective gene.

74. The method of Claim 73, wherein the DNA is a gene library.

75. The method of Claim 73, wherein the nucleic acid probe further comprises degenerate oligonucleotides derived from the amino acid sequence of the product of the cell death-protective gene.

76. The method of Claim 70, wherein step a) comprises the steps of:

a) combining nucleic acid with primers comprising portions of the cell death-protective gene under conditions suitable for polymerase chain reaction; and

b) detecting specific DNA amplification, wherein specific DNA amplification produces a structurally similar gene, or portion,

thereby identifying a gene which is structurally similar to a cell death-protective gene.

77. The method of Claim 76, wherein the primers further comprise degenerate oligonucleotides derived from the amino acid sequence of the product of the cell death-protective gene.

78. The method of Claim 70, wherein step a) comprises the steps of:

a) combining an expression gene library with an antibody which binds specifically with the protein encoded by the cell death-protective gene under conditions suitable for specific antibody-antigen binding of the antibody to antigens expressed from the gene library; and

b) detecting specific antibody-antigen binding, wherein specific antibody-antigen binding indicates that a structurally similar gene is present in the expression gene library,

thereby identifying a gene which is structurally similar to a cell death-protective gene.

79. The method of Claim 70, wherein step a) comprises searching a database of genes for a nucleotide sequence which is similar to the nucleotide sequence of the cell death-protective gene, thereby identifying a gene which is structurally similar to the cell death-protective gene.

80. The method of Claim 70, wherein step a) comprises searching a database of proteins for an amino acid sequence which is similar to the amino acid sequence of the protein encoded by the cell death-protective gene, thereby identifying a gene which is structurally similar to the cell death-protective gene.

81. The method of Claim 70, wherein step b) comprises the steps of:

a) using the structurally similar gene and a nematode which lacks the activity of the ced-9 gene to produce a transgenic nematode; and

b) determining in said transgenic nematode a decrease in cell deaths which occur during the development of the nontransgenic nematode, wherein a decrease in cell deaths indicates that the structurally similar gene protects cells in which it functions from cell death.

82. The method of Claim 70, wherein step b) comprises the steps of:

a) introducing the structurally similar gene into cultured mammalian cells to produce transfected cells which express the gene; and

b) determining a decrease in cell deaths among the transfected cells under conditions which induce cell death, wherein a decrease in cell deaths indicates that the gene protects cells in which it functions from cell death.

83. A bioassay for identifying a gene which has cell death-protective activity, comprising the steps of:

a) using DNA and a nematode which lacks the activity of the ced-9 gene to produce a transgenic nematode; and

b) determining in said transgenic nematode a decrease in cell deaths which occur during the development of the nontransgenic nematode, wherein a decrease in cell deaths indicates the activity of a cell death-protective gene in the DNA,

thereby identifying a gene which has cell death-protective activity.

84. The bioassay of Claim 83, wherein the nematode underexpresses or expresses inactivated ced-9.

85. The bioassay of Claim 83, wherein the DNA is from an organism other than a nematode.

86. The bioassay of Claim 83, wherein the DNA is an expression gene library.

87. Substantially pure nucleic acid which is a cell death-protective gene identified by the bioassay of Claim 83.

88. Substantially pure nucleic acid which is a mutated cell death-protective gene identified by the bioassay of Claim 87.

89. A bioassay to identify a mutation in a cell death-protective gene which alters the activity of the gene, comprising the steps of:

a) using a cell death-protective gene having a mutation and a nematode which lacks ced-9 activity to produce a transgenic nematode; and

b) comparing cell deaths which occur during the development of the transgenic nematode having the mutated gene with those which occur in a transgenic nematode having a non-mutated gene, wherein a difference in cell deaths indicates that the mutation alters the activity of the cell death-protective gene,

thereby identifying a mutation in a cell death-protective gene which alters the activity of the gene.

90. Substantially pure nucleic acid which is a cell death-protective gene having a mutation identified by the bioassay of Claim 89.

91. A bioassay for identifying an agent which mimics the activity of a cell death-protective gene, comprising the steps of:

a) introducing an agent into a nematode which lacks the activity of ced-9; and

b) detecting a decrease in cell deaths which occur in the nematode, wherein a decrease indicates that the agent mimics the activity of a cell death-protective gene,

thereby identifying an agent which mimics the activity of a cell death-protective gene.

92. The bioassay of Claim 91, wherein the nematode underexpresses or expresses inactivated ced-9.

93. The bioassay of Claim 91, wherein the agent is introduced into the nematode by a method selected from: microinjection, diffusion, or ingestion.

94. An agent identified by the bioassay of Claim 91.

95. A bioassay for identifying an agent which affects the activity of a cell death-protective gene, comprising the steps of:

a) introducing an agent into a nematode which expresses a cell death-protective gene; and

b) detecting a change in the pattern of cell deaths which occur in the development of the nematode, wherein a change indicates that the agent affects the activity of the cell death-protective gene,

thereby identifying an agent which affects the activity of a cell death-protective gene.

96. The bioassay of Claim 95, wherein the cell death-protective gene is ced-9.

97. The bioassay of Claim 95, wherein the cell death-protective gene is not ced-9 and the nematode is a transgenic nematode produced from said cell death-protective gene and a nematode which lacks ced-9 activity.

98. The bioassay of Claim 97, wherein the cell death-protective gene is bcl-2.

99. The bioassay of Claim 95, wherein the nematode overexpresses or underexpresses the cell death-protective gene.

100. The bioassay of Claim 95, wherein the nematode expresses an inactivated or constitutively activated form of the cell death-protective gene.

101. The bioassay of Claim 95, wherein the nematode underexpresses or expresses inactivated ced-9.

102. An agent identified by the method of Claim 95.

103. The agent of Claim 102, which is selected from the group consisting of:

a) single stranded nucleic acid comprising all or a portion of the antisense sequence of a cell death-protective gene which is complementary to the mRNA encoded by the gene; and

b) DNA encoding a).

104. The agent of Claim 102, which is a gene which is not a cell death-protective gene.

105. The agent of Claim 102, which is a mutated cell death-protective gene which does not protect cells from cell death and which antagonizes the activity of cell death-protective genes which function.

106. A bioassay for identifying a cell death-protective gene, comprising the steps of:

a) introducing DNA into cultured mammalian cells to produce transfected cells which express gene(s) in the DNA; and

b) detecting cell deaths among the transfected cells under conditions which induce cell death, wherein a decrease in cell deaths indicates the activity of a cell death-protective gene in the DNA,

thereby identifying a cell death-protective gene.

107. The bioassay of Claim 106, wherein the DNA is an expression gene library.

108. Substantially pure nucleic acid which is a gene identified by the method of Claim 106.

109. A bioassay for identifying a mutation in a cell death-protective gene which alters the activity of the gene, comprising the steps of:

a) introducing a cell death-protective gene having a mutation into cultured mammalian cells to produce transfected cells which express the mutated gene; and

b) comparing cell deaths which occur among the transfected cells under conditions which induce cell death to cell deaths which occur among cells transfected with the non-mutated gene, wherein a difference in cell deaths indicates that the mutation alters the activity of the gene,

thereby identifying a mutation which alters the activity of a cell death-protective gene.

110. Substantially pure nucleic acid which is a cell death-protective gene having a mutation identified by the bioassay of Claim 109.

111. A bioassay for identifying an agent which mimics the activity of a cell death-protective gene, comprising the steps of:

a) exposing cultured mammalian cells to an agent under conditions which induce cell death and which are appropriate for activity of the agent; and

b) detecting cell deaths in said exposed cells, wherein a decrease in cell deaths indicates that the agent protects cells from cell death,

thereby identifying an agent which mimics the activity of a cell death-protective gene.

112. An agent identified by the bioassay of Claim 111.

113. A bioassay for identifying an agent which affects the activity of a cell death-protective gene, comprising the steps of:

a) exposing cultured mammalian cells which are protected by the activity of a cell death-protective gene from conditions which induce cell death to an agent under conditions which are appropriate for activity of the agent; and

b) detecting cell deaths in said exposed cells, wherein a change in cell deaths indicates that the agent affects the activity of the gene,

thereby identifying an agent which affects the activity of a cell death-protective gene.

114. An agent identified by the bioassay of Claim 113.

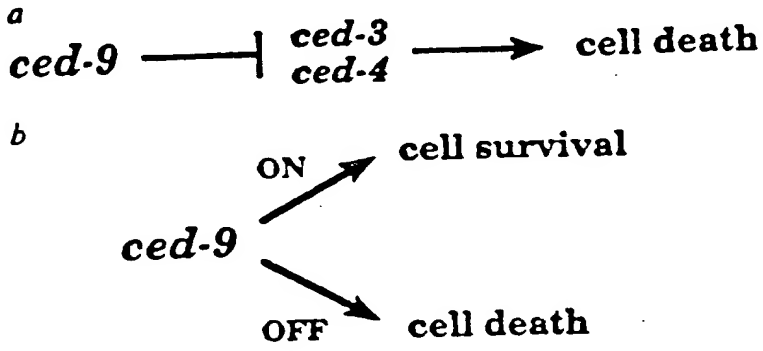
a
ced-9
ced-3
ced-4
cell death
b
ON
cell survival
ced-9
OFF
cell death

FIGURE 1

```
ATCGATAGTC GTCACCAAAT GGATTTTCCG ATTTCTCACT AGTCCATGGC TCACAATTTA      60
CAAAATCTCG AGAAAAGAAA GGATGCAAGG AGTATGAAGA GGTTCCGAAT CTAAATATTT     120
TAATTTAAAA AAATCAATTT CGAATTGAAA TTCAACTCCT ACTCGTTTTG AAAATGCCAA     180
TCCTTTAAGT AAACTTCTGG ATCGCCCATT TCTTCCAGAA ATTCCTTCAA AGTAGTGGTT     240
TTGTACTGAT TTCCTCCGCA AAGAATAGGA ACTTTCGAAT CTCCTGGAGC GAAACGGGAT     300
TTTSATAACA AAAAACTATC CAGACAAACC ATAGGACTTT TTCAAATATT CCTTATTTGG     360
CTGTCCATTT GGAAGCACCC AATCTTTAAC GCTGTCCAGC CAGAAGTGCT CCACTCGCCA     420
AGGATAAAAG GCTCATTTTT GAAGCCGAAT TTTACTAAAA TCTCTAGCCA TGGAGTCGAT     480
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ATTCAAAGAA AATCACAGAA AATGCAACAA AAAAAACAAA AAAAGAACAA AAAACAAGTC     600
GAAAAGTGCG CCCGGGTCGT TTGCTGACGC ATCTCTTCAA ACGAGACGCG CTGCTGGCGA     660
CTTCTCGTGC CCTGTGCGTG CATTTCCGCA ACAAAATTCA ACACTTGTTT TGAAACGCAC     720
CGCCCTGTTT CTTTTTTCAA TTTTGATAAG AAAATCAGCA TTGTTTCAGG ATGATTAACA     780
TTCCAACTGC GATTCTGTGC CGCTTGGGCG CCAGATCGTC GATTTCCCGC TCCTTTGGAA     840
CATCGATCGT CACCAAGGTG GGGATTTTTT GAATTTTTCC GTGAAAATTG TTGATTTTTT     900
GTGTACGCAT GAAGGAGAAA TGTATAACAG ACACATTCTT TTCAATTAAT TATTTATAAT     960
ATTCACAGTC CGAGGCAAAG ACGCCAATCC AGAAGTTCGG ATGGGAATAC CTGTTGAAGC    1020
AGCGCTCCAA GAATCGCCCA ATCGCTCCAC ATCTCACCGT CTACCAGCCA CAATTGACCT    1080
GGATGCTCTC CGGATTCCAT AGAATCAGCG GTTGTGTAAT GGCCGGAACC CTTCTCGTCG    1140
GAGGAATCGG ATTCGCAGTT TTGCCGTTCG ATTTCACCGC TTTTGTGGAT TTCATCCGTA    1200
GCTGGAACTT ACCATGCGCG GTGACCGCTG TCTTCAAGTA CATCATTGCT TTCCCCATCA    1260
TTTTCCATAC TCTTAACGGA ATTCGCTTCT TAGGATTCGA TTTGGCTAAG GGAGTCAATA    1320
ATGTTGGACA GGTAGGAGTT GAAATTATTA ATTTAATTGT TTTAAAATAA AAATTAATTT    1380
TCAGATCTAC AAATCGGGAT ATCTCGTATC TGGACTTTCG GCTATTCTTG CTCTCGCCAT    1440
TGTCTTCAAC TCTTGCCAGA ACAAGAGCAA CAAGACTGCC TAGGCACAGA TGCTCCGCCT    1500
TCTTTTTTCT TACTCCGCCC CAGCCCTCGA CAATTCTCGT CAATTTACTT TTACCGTTGA    1560
TTTCTTCGAT TTTCTCTCTT TTCCGTAGAT TTACCTCTCC TCTTCGTTTT TTTTTCTCTG    1620
TCTAGAATGT ATATTATGAT TATGAAAACG AATAAAAATT TTAGATGACA CGCTGCACGG    1680
CGGACAACTC GCTGACGAAT CCGGCGTATC GGCGACGAAC GATGGCGACT GGCGAGATGA    1740
AGGAGTTTCT GGGGATAAAA GGCACAGAGC CCACCGATTT TGGAATCAAT AGTGATGCTC    1800
AGGACTTGCC ATCACCGAGT AGGCAGGCTT CGACGCGAAG AATGTCCATC GGAGAGTCAA    1860
```

FIGURE 2(a)

TTGATGGAAA AATCAATGAT TGGGAAGAGC CAAGGCTTGA TATCGAGGGA TTTGTGGTAA 1920

TTTTTTAATT TTTTTTTGTA AATAAAATTT CCTGCTGCTT CCAGGTCGAC TATTTCACGC 1980

ACCGAATCCG GCAAAACGGA ATGGAATGGT TTGGAGCACC GGGATTGCCG TGTGGAGTGC 2040

AACCGGAGCA CGAAATGATG CGAGTTATGG GAACGATATT CGAGAAGAAG CACGCGGAAA 2100

ATTTTGAGAC CTTCTGTGAG CAGCTGCTCG CAGTGCCCAG AATCTCATTT TCACTGTATC 2160

AGGATGTGGT TCGGACGGTT GGAAATGCAC AGACAGATCA ATGTCCAATG TCTTATGGAC 2220

GTTTGGTAAG GGAGAAAATA CTGAAAAAAA GTTTGCAAAA ATTCGAAAAT TCGCCAGAAA 2280

GGTGGCAGAA AAAACATTTG CAAAAATTGT TTGTTTTCCT TCAGGAAATC AGCAAAACTT 2340

GGTCAAAAAT AGCCCAATTA TGTGTCTTTT TTGAAAGTTT TCCATTAAAA AACCACGAAT 2400

TTTGATCCCG GATTGTAATT TTTTTTGTTG ATAAATTAGC AGAAAACTTT ACGAATTCGA 2460

TTAAAAACGT TATTTTCTAT TCGAATATTT TTAAAGCATA TTTTCCTTGA TTTGTATTTG 2520

CGAAAAAGAT CTGCTGATTT ATCAAAAATC GGTTTTTAAA TGTAAAATTT GTGGAAAATA 2580

CATTAAAATT CGATTTTTGA ACTTTTTTCT TCGAAAAACA GGTTTTTCTG CTGATTTGCT 2640

GAACGAAAAA CCCCAAAAAT TCAATTTTCG AACATTAAAA ACCAGAAAAA TCGTTTTTTT 2700

AAGCTTAATT TTCCGCCAGA AATGAACGAA TTAAATTGCA AATTTCTAAT TTTCAGATAG 2760

GTCTAATCTC GTTCGGCGGT TTCGTAGCTG CAAAAATGAT GGAATCCGTG GAACTGCAGG 2820

GACAAGTGCG AAACCTCTTC GTTTACACAT CGCTGTTCAT CAAAACGCGG ATCCGCAACA 2880

ACTGGAAGGA ACACAATCGG AGCTGGGTAA GGAGTATTTG CATAGACATT AGAAGTCAAT 2940

ATCCCCCTTT CCCTAGTACC CTTGACTTCC CGGGGTGTTG GTAAGCCGAT AATTACAGGG 3000

TTCGGTAGCC TCTTGGGGGG ACAGCTGGAA ACATATTCAA GTATATTACT GTTTATGATA 3060

ATGTTATTGT TACGGGAATA CAAAATTCGC AGAATGCTAT TTCACAACAT ATTTGACGCG 3120

CAAAATATCC AGTAGAGAAA ACTACAGTAA TTCTTTAAAT TTTTAAAATT TTTACAATTA 3180

AAGAAAATAA CCACTAATCA AAAGAAATTA ATTTCAAAAA TCGAGCCCGT AAATCGACTA 3240

CAGTAGGCAT TTAAAGAATT ACTGTAGTTT TCGCTACGAG ATATTTCCGC CTCAAATATG 3300

TTGTGAAATA CGCATTCACG GATTTTTGTG TTCCCCGGAA TATGCTCTAA AGCATTATTT 3360

GTGAAAATAA AAAATCAAGA AAAAAATTGC AGGACGACTT CATGACACTC GGAAAACAAA 3420

TGAAAGAGGA CTACGAACGA GCAGAAGCTG AAAAAGTGGG ACGCCGGAAG CAGAACAGAC 3480

GGTGGTCGAT GATTGGCGCT GGAGTAACAG CTGGAGCCAT TGGAATCGTT GGAGTCGTCG 3540

TGTGTGGGCG GATGATGTTC AGCTTGAAGT AACGTATTCA ATTTGTGTAA ATAATTAATT 3600

TATGTACAAC TCCTTACATT TGAATCTCAT TTTTGCTCAC TGATTCTCTC ATCCTTTGAA 3660

CTGGAAGAAG TGGGAAAGCT AGGCCACAAA TTACGGCTCT CTGTGTCGAT TTACGATTTT 3720

FIGURE 2(b)

ACTGCAATTT TTTCCGATTG CCTTTTTTTT TGGCCAAACC CTACTTCCGC GTAATATCAA 3780

CTTTTCCGTG TTCTGTACAT TTCGTCAAAA ACCCTGAAAC CCTAACTTTT CTCGCCGTGG 3840

CCTAGCCTCC CGCTTCTCTT CCACATTTCC AAAGTACCCC TGTATCTCAA TAATTCATCT 3900

TCACTTTAAC TGTCTCTTTT CGTGTGGCCT CTTCCAACTC CCCCCAAATT CCTGTACGCG 3960

TACGCGACTT TGTATTTATT TTTTTCAAAT TGTTTTCTCT CTACAACAAC AAAAAAAACG 4020

GTTCTTTTAT TCAACCCTTT TTTCGGAACG AAACTGCAAT TTTGATAATA GGCGTGCGCA 4080

AGAGAATCCG GTTTTCATTT TCGCCATCAC GTCATCCAAA AAAGTTTAGT AGGAAAATAT 4140

CATTTTTTAA TATAATGATT CATCTTTCTC GCCTCTTCTG TCTCGAGACG ACGGTCAATT 4200

CGATGGCCTT GAATTTTTCG AAAACAAAAA TGTTTTTGTT TAGTGTAAAC GATCCCCCCG 4260

CCTTATCGCT GTTTCACCAT CAGATAGGCT CCGCCATTTG ATTCCCTTGA ATTTTGTCGG 4320

TATATAAAAC AAAAAACGTT AGTGCACGAT TCAAAAAACA ACAATGCGTG CTTTACTATT 4380

CACCTCTGTT GTTCTTTTGG CTTTGGCTTT TGTTGAGGCA AAGAAGCAGA CTATCACTGT 4440

CAAGGGTACA ACTATTTGTA ATAAGAAGAG AATTCAGGMG RAGGTTACCT TTGGGAGAAA 4500

GATACTCGTG AGTTTTCAGT CTTGTTTAGC TTGAAACGGC TTAAAAAGGA CTAAAAAGGC 4560

CTAAAAATTG AAGTTTTCCA CCTGTTTTCA AAAGAAAGCC GAATTGCACA GCTTTACACG 4620

AGATTTCTCA ATAATTTGTA TTTGAAATTT TCATATTCAT CCCCAAACGT TCTTTACACG 4680

AAATTTTGCG ATTTTTGAGC TTAAAATACG ATACCTGGTC TCGACACGAA ACATTTTTGT 4740

TAAATTCAAA AAGATGTGCG CCTTTAAAGA GTGCTGTAGT TTGAAACTTC TGTTGTTGCG 4800

GACTTTTCAT CGATTTTTCG TAGCGTTTTT TTATAAGAAA AATGTATTTA TTTATTCAAA 4860

AATTTAATTT TACCGAATCG CGAAAAACAA AATGAAGAAC ACCGATAAAA ATATCGCAGC 4920

AACAATAGTT TGAAATTACA GTACTCTTTT AAGGNGNNCA CATTTCCTAT ATTTCACACA 4980

AACTTGTCGT GTCGNNNCNG GGTATCGTCA TTTTGATGCA GAAATCAAGA AAATTGCATA 5040

TATGTTCAAA AAACCACAAT TATGGCGAAT TTCAAGCTTG AAACGAAAAT TCAGGAAATT 5100

CTAAAAATTA AAAAAAAATC ATTCGAAATG TGAAATTTGA TATTCAACTT GAAGTCCATA 5160

TGGCAAATTT CGTCTATTCC GNNNTTCGAN NATTTTGTTC CACGTGGCCG CGAAAAGAGA 5220

AAGCACGANN ACTGATTTCT GGCAATTTTT TCCTGTACCG TGTCAATTAT TTGAAACTCT 5280

AATAAGCTGG TATTTTTCTG CTATTGACAA CTAACTGAAT CCATAATTTG CAATTATAAT 5340

ATTGACTTTT GATGTGTGGC TTAGAAAAAA AAAACCAAAA ACCTCATCTA GCTTTAGGCT 5400

GCCAATATAT TCCTAGGACA TATAAAAAAC CCTTAAAATT CTCTGCAACA CCTACAAGCT 5460

ATCAAACGTA CTATTAGTAT TCAATTTTCC AGTCGACCCC GATGACAAGC TCGCCTCAAT 5520

GCAATCGAAC AAAGAAGGAG AGTTCTCACT TACCGGATCC GACGACGAGA TCACCTCAAT 5580

FIGURE 2(c)

TCTCTCCATA CCTCATAATC ACCCACAACT GCAACGTGAA GAAGGCCGGA TGCAAGCGTG 5640

TTTCAGAGTA TTTGATTCCA AAGGAGAAGA TCGGTGGAAC CTATGATATG ACATACGTCA 5700

CTCTTGATAT TCTTTCCGCT AAAGACAAGG AGAAGTGCTA AGAAAATGTT TTTTTTGTTT 5760

GGTTTGCTTG TTTGGAAGGG AAGGACTTTC TATCTCTTTT AATTCAACAA TAAACTATTG 5820

GAAAACCGTT GAAATTTTAA CCTTGAACTG TAAGAAAAGT TGCGTGATTA TGTTGACAAT 5880

TTTGCCAAGT ATATCTTTGT GGATATCACA ATAAACGAAG TCAAAGCACG AAATATTACG 5940

GAAACACAAA ATTAATGAGA ATGCGCAACA TATTTGACCG CAAAATATCT CGTAGCGAAA 6000

CTACAGTAAT TCTTCAAAAG ACTACTGTAG CGCTGTGTCG ATTTACGAGC TCGATTTTTG 6060

AAATGAATCA GACTAGAAGA AAAGGAGGAA AATATTGAAC ATCAATTGAA CATCAATTCA 6120

AAAAGTCGAA CCCTTGACTA CAGTAGTCTT CTAAAGAATT ACTGTAGTTT TCGCTACGAG 6180

ATATTTTGNG NGTCAAATAT GTTGNGCAAT ACGCATCCTC AGAATTGTGT GTTCTCGTAA 6240

TGTCTTGAAA ATTTTCCATT TCAACATCAA ATAAGCAAAT CTAAAAATGT GGGTTCTGCA 6300

GCGACCACTA TGACTGTGAT CGTGGCAAGA CCCACTCAGA AAACTACGTG TTCCTTTAAA 6360

CAAATACATT TTTAAGTATT GTAGGTATAA AAATTGTTGG CTAGCAGTCT AGGCTGCCTT 6420

TTTCAGTCGA CAAACTTCTA ATTTAATCGG CGGGTCTTCA AAAAGTCGTT TCTTTGAAAA 6480

TATAAAGCTT TATATATTTA TATATTAAAA ATTTTGATTA CATGATATCA AAAGCGACTA 6540

GTTTGTATAA AAATTATCAA 6560

FIGURE (2d)

```
TTT GAG ATG ACA CGC TGC ACG GCG GAC AAC TCG CTG ACG AAT CCG GCG          48
        Met Thr Arg Cys Thr Ala Asp Asn Ser Leu Thr Asn Pro Ala
          1               5                  10

TAT CGG CGA CGA ACG ATG GCG ACT GGC GAG ATG AAG GAG TTT CTG GGG          96
Tyr Arg Arg Arg Thr Met Ala Thr Gly Glu Met Lys Glu Phe Leu Gly
 15                  20                  25                  30

ATA AAA GGC ACA GAG CCC ACC GAT TTT GGA ATC AAT AGT GAT GCT CAG         144
Ile Lys Gly Thr Glu Pro Thr Asp Phe Gly Ile Asn Ser Asp Ala Gln
                    35                  40                  45

GAC TTG CCA TCA CCG AGT AGG CAG GCT TCG ACG CGA AGA ATG TCC ATC         192
Asp Leu Pro Ser Pro Ser Arg Gln Ala Ser Thr Arg Arg Met Ser Ile
              50                  55                  60

GGA GAG TCA ATT GAT GGA AAA ATC AAT GAT TGG GAA GAG CCA AGG CTT         240
Gly Glu Ser Ile Asp Gly Lys Ile Asn Asp Trp Glu Glu Pro Arg Leu
          65                  70                  75

GAT ATC GAG GGA TTT GTG GTC GAC TAT TTC ACG CAC CGA ATC CGG CAA         288
Asp Ile Glu Gly Phe Val Val Asp Tyr Phe Thr His Arg Ile Arg Gln
      80                  85                  90

AAC GGA ATG GAA TGG TTT GGA GCA CCG GGA TTG CCG TGT GGA GTG CAA         336
Asn Gly Met Glu Trp Phe Gly Ala Pro Gly Leu Pro Cys Gly Val Gln
 95                  100                 105                 110

CCG GAG CAC GAA ATG ATG CGA GTT ATG GGA ACG ATA TTC GAG AAG AAG         384
Pro Glu His Glu Met Met Arg Val Met Gly Thr Ile Phe Glu Lys Lys
                115                 120                 125

CAC GCG GAA AAT TTT GAG ACC TTC TGT GAG CAG CTG CTC GCA GTG CCC         432
His Ala Glu Asn Phe Glu Thr Phe Cys Glu Gln Leu Leu Ala Val Pro
            130                 135                 140

AGA ATC TCA TTT TCA CTG TAT CAG GAT GTG GTT CGG ACG GTT GGA AAT         480
Arg Ile Ser Phe Ser Leu Tyr Gln Asp Val Val Arg Thr Val Gly Asn
        145                 150                 155

GCA CAG ACA GAT CAA TGT CCA ATG TCT TAT GGA CGT TTG ATA GGT CTA         528
Ala Gln Thr Asp Gln Cys Pro Met Ser Tyr Gly Arg Leu Ile Gly Leu
    160                 165                 170

ATC TCG TTC GGC GGT TTC GTA GCT GCA AAA ATG ATG GAA TCC GTG GAA         576
Ile Ser Phe Gly Gly Phe Val Ala Ala Lys Met Met Glu Ser Val Glu
175                 180                 185                 190

CTG CAG GGA CAA GTG CGA AAC CTC TTC GTT TAC ACA TCG CTG TTC ATC         624
Leu Gln Gly Gln Val Arg Asn Leu Phe Val Tyr Thr Ser Leu Phe Ile
                195                 200                 205

AAA ACG CGG ATC CGC AAC AAC TGG AAG GAA CAC AAT CGG AGC TGG GAC         672
Lys Thr Arg Ile Arg Asn Asn Trp Lys Glu His Asn Arg Ser Trp Asp
            210                 215                 220

GAC TTC ATG ACA CTC GGA AAA CAA ATG AAA GAG GAC TAC GAA CGA GCA         720
Asp Phe Met Thr Leu Gly Lys Gln Met Lys Glu Asp Tyr Glu Arg Ala
        225                 230                 235

GAA GCT GAA AAA GTG GGA CGC CGG AAG CAG AAC AGA CGG TGG TCG ATG         768
Glu Ala Glu Lys Val Gly Arg Arg Lys Gln Asn Arg Arg Trp Ser Met
    240                 245                 250
```

FIGURE 3(a)

```
ATT GGC GCT GGA GTA ACA GCT GGA GCC ATT GGA ATC GTT GGA GTC GTC        816
Ile Gly Ala Gly Val Thr Ala Gly Ala Ile Gly Ile Val Gly Val Val
255                 260                 265                 270

GTG TGT GGG CGG ATG ATG TTC AGC TTG AAG TAACGTATTC AATTTGTGTA         866
Val Cys Gly Arg Met Met Phe Ser Leu Lys
                275                 280

AATAATTTAAT TTATGTACAA CTCCTTACAT TTGAATCTCA TTTTKGCTCA CTGATTCTCT     926

CATCCTTTGA ACTGGAAGAA GTGGGAAAGC TAGGCCACAA ATTACGGCTC TCTGTGTCGA     986

TTTACGATTT TACTGCAATT TTTTCCGATT GCCTTTTTTT TTGGCCAAAC CCTACTTCCG    1046

CGTAATATCA ACTTTTCCGT GTTCTGTACA TTTCGTCAAA AACCCTGAAA CCCTAACTTT    1106

TCTCGCCGTG GCCTAGCCTC CCGCTTCTCT TCCACATTTC CAAAGTACCC CTGTATCTCA    1166

ATAATTCATC TTCACTTTAA CTGTCTCTTT TCGTGTGGCC TCTTCCAACT CCCCCCAAAT    1226

TCCTGTACGC GTACGCGACT TTGTATTTAT TTTTTTCAAA TTGTTTTCTC TCTACAACAA    1286

CAAAAAAAAC GGTTCAAAAA AAAAAAAAA                                      1315
```

FIGURE 3(b)

B30 ORF 920610 Sequence

10	20	30	40	50	
1234567890	1234567890	1234567890	1234567890	1234567890	
MTRCIADNSL	TNPAYRRRIM	ATGEMKEFLG	IKGTEPTDFG	INSDAQDLPS	50
PSRQASTRRM	SIGESIDGKI	NDWEEPRLDI	EGFVVDYFTH	RIRQNGMEWF	100
GAPGLPCGVQ	PEHEMMRVMG	TIFEKKHAEN	FETFCEQLLA	VPRISFSLYQ	150
DVVRIVGNAQ	TDQCPMSYGR	LIGLISFGGF	VAAKMMESVE	LQGQVRNLFV	200
YTSLFIKTRI	RNNWKEHNRS	WDDFMTLGKQ	MKEDYERAEA	EKVGRRKQNR	250
RWSMIGAGVT	AGAIGIVGVV	VCGRMMFSLK			280

FIGURE 4

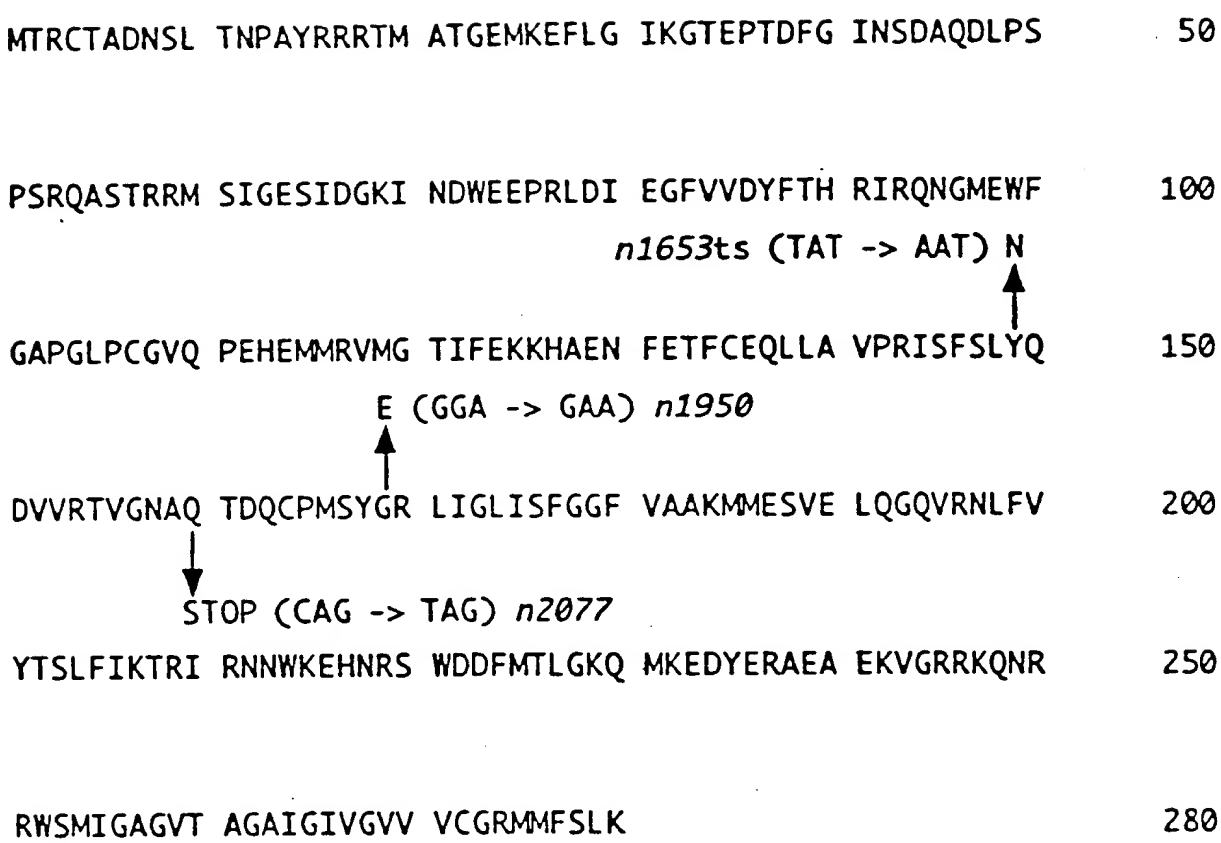
MTRCTADNSL TNPAYRRRTM ATGEMKEFLG IKGTEPTDFG INSDAQDLPS 50
PSRQASTRRM SIGESIDGKI NDWEEPRLDI EGFVVDYFTH RIRQNGMEWF 100
n1653ts (TAT -> AAT) N
GAPGLPCGVQ PEHEMMRVMG TIFEKKHAEN FETFCEQLLA VPRISFSLYQ 150
E (GGA -> GAA) n1950
DVVRTVGNAQ TDQCPMSYGR LIGLISFGGF VAAKMMESVE LQGQVRNLFV 200
STOP (CAG -> TAG) n2077
YTSLFIKTRI RNNWKEHNRS WDDFMTLGKQ MKEDYERAEA EKVGRRKQNR 250
RWSMIGAGVT AGAIGIVGVV VCGRMMFSLK 280

FIGURE 5

Gap Weight:	3.000	Average Match:	0.540
Length Weight:	0.100	Average Mismatch:	-0.396
Quality:	89.8	Length:	298
Ratio:	0.376	Gaps:	11
Percent Similarity:	47.059	Percent Identity:	23.077

```
  1 ...MTRCTADN.........SLTNPAYRRRTMATGEMKEFLGIKGTEPT  37
      .|..  ||          .|...:| :   ..|:: .   .  :..|.
  1 MAHAGRTGYDNREIVMKYIHYKLSQRGYEW...DAGDVGAAPPGAAPAPG  47

 38 DFGINSDAQDLPSPSRQASTRRMSIGESIDGKINDWEEPRLDIEGFVVDY  87
      |: ..:   . |.:||:: .|  .:...  .
 48 IFSSQPGHTPHPAASRDPVARTSPLQTPAAPGAA.............. 81

 88 FTHRIRQNGMEWFGAPGLPCGVQPEHEMMRVMGTIFEKKHAKNFETFCEQ 137
                 ::|:|.....  |  :|  |.  |.:::.  :|..:::.|
 82 ............AGPALSPVPPVVHLALRQAGDDFSRRYRGDFAEMSSQ 118

138 LLAVPRISFSLYQDVVRTVGNAQTDQCPMSYGRLIGLISFGGFVAAKMME 187
    |  .|.. : :..|| .:  ..      .:.:|||:::::.|||.:.   :|
119 LHLTPFTARGRFATVVEELFRD.....GVNWGRIVAFFEFGGVMC...VE 160
    conserved residue mutated in n1950

188 SV..ELQGQVRNLFVYTSLFIKTRIRNNWKEHNRSWDDFMTL.GKQMKE. 233
    ||  |: . | |: :: . ::. :: :.| :.| :||.|:.| |.  |:.
161 SVNREMSPLVDNIALWMTEYLNRHL.HTWIQDNGGWDAFVELYGPSMRPL 209

234 .DYERAEAEKVGRRKQNRRWSMIGAGVTAGAIGIVGVVVCGRMMFSLK 280
     |:.: . ..:          .:.:::||.:| ||.            :| |
210 FDFSWLSLKTL......LSLALVGACITLGAY.............LSHK 239
```

FIGURE 6

```
  1  GCGCCCGCCC CTCCGCGCCG CCTGCCCGCC CGCCCGCCGC GCTCCCGCCC
 51  GCCGCTCTCC GTGGCCCCGC CGCGCTGCCG CCGCCGCCGC TGCCAGCGAA
101  GGTGCCGGGG CTCCGGGCCC TCCCTGCCGG CGGCCGTCAG CGCTCGGAGC
151  GAACTGCGCG ACGGGAGGTC CGGGAGGCGA CCGTAGTCGC GCCGCCGCGC
201  AGGACCAGGA GGAGGAGAAA GGGTGCGCAG CCCGGAGGCG GGGTGCGCCG
251  GTGGGGTGCA GCGGAAGAGG GGGTCCAGGG GGGAGAACTT CGTAGCAGTC
301  ATCCTTTTTA GGAAAAGAGG GAAAAAATAA AACCCTCCCC CACCACCTCC
351  TTCTCCCCAC CCCTCGCCGC ACCACACACA GCGCGGGCTT CTAGCGCTCG
401  GCACCGGCGG GCCAGGCGCG TCCTGCCTTC ATTTATCCAG CAGCTTTTCG
451  GAAAATGCAT TTGCTGTTCG GAGTTTAATC AGAAGACGAT TCCTGCCTCC
```

FIGURE 7 (a)

```
 501  GTCCCCGGCT CCTTCATCGT CCCATCTCCC CTGTCTCTCT CCTGGGGAGG
 551  CGTGAAGCGG TCCCGTGGAT AGAGATTCAT GCCTGTGTCC GCGCGTGTGT
 601  GCGCGCGTAT AAATTGCCGA GAAGGGGAAA ACATCACAGG ACTTCTGCGA
 651  ATACCGGACT GAAAATTGTA ATTCATCTGC CGCCGCCGCT GCCAAAAAAA
 701  AACTCGAGCT CTTGAGATCT CCGGTTGGGA TTCCTGCGGA TTGACATTTC
 751  TGTGAAGCAG AAGTCTGGGA ATCGATCTGG AAATCCTCCT AATTTTTACT
 801  CCCTCTCCCC CCGACTCCTG ATTCATTGGG AAGTTTCAAA TCAGCTATAA
 851  CTGGAGAGTG CTGAAGATTG ATGGGATCGT TGCCTTATGC ATTTGTTTTG
 901  GTTTTACAAA AAGGAAACTT GACAGAGGAT CATGCTGTAC TTAAAAAATA
 951  CAAGTAAGTC TCGCACAGGA AATTGGTTTA ATGTAACTTT CAATGGAAAC
1001  CTTTGAGATT TTTTACTTAA AGTGCATTCG AGTAAATTTA ATTTCCAGGC
1051  AGCTTAATAC ATTGTTTTTA GCCGTGTTAC TTGTAGTGTG TATGCCCTGC
1101  TTTCACTCAG TGTGTACAGG GAAACGCACC TGATTTTTTA CTTATTAGTT
1151  TGTTTTTTCT TTAACCTTTC AGCATCACAG AGGAAGTAGA CTGATATTAA
1201  CAATACTTAC TAATAATAAC GTGCCTCATG AAATAAAGAT CCGAAAGGAA
1251  TTGGAATAAA AATTTCCTGC GTCTCATGCC AAGAGGGAAA CACCAGAATC
1301  AAGTGTTCCG CGTGATTGAA GACACCCCCT CGTCCAAGAA TGCAAAGCAC
1351  ATCCAATAAA ATAGCTGGAT TATAACTCCT CTTCTTTCTC TGGGGGCCGT
1401  GGGGTGGGAG CTGGGGCGAG AGGTGCCGTT GGCCCCCGTT GCTTTTCCTC
1451  TGGGAAGGAT GGCGCACGCT GGGAGAACGG GGTACGACAA CCGGGAGATA
1501  GTGATGAAGT ACATCCATTA TAAGCTGTCG CAGAGGGGCT ACGAGTGGGA
1551  TGCGGGAGAT GTGGGCGCCG CGCCCCCGGG GGCCGCCCCC GCACCGGGCA
1601  TCTTCTCCTC CCAGCCCGGG CACACGCCCC ATCCAGCCGC ATCCCGCGAC
1651  CCGGTCGCCA GGACCTCGCC GCTGCAGACC CCGGCTGCCC CCGGCGCCGC
1701  CGCGGGGCCT GCGCTCAGCC CGGTGCCACC TGTGGTCCAC CTGGCCCTCC
1751  GCCAAGCCGG CGACGACTTC TCCCGCCGCT ACCGCGGCGA CTTCGCCGAG
1801  ATGTCCAGCC AGCTGCACCT GACGCCCTTC ACCGCGCGGG GACGCTTTGC
1851  CACGGTGGTG GAGGAGCTCT TCAGGGACGG GGTGAACTGG GGGAGGATTG
1901  TGGCCTTCTT TGAGTTCGGT GGGGTCATGT GTGTGGAGAG CGTCAACCGG
```

FIGURE 7 (b)

```
1951  GAGATGTCGC CCCTGGTGGA CAACATCGCC CTGTGGATGA CTGAGTACCT
2001  GAACCGGCAC CTGCACACCT GGATCCAGGA TAACGGAGGC TGGGATGCCT
2051  TTGTGGAACT GTACGGCCCC AGCATGCGGC CTCTGTTTGA TTTCTCCTGG
2101  CTGTCTCTGA AGACTCTGCT CAGTTTGGCC CTGGTGGGAG CTTGCATCAC
2151  CCTGGGTGCC TATCTGAGCC ACAAGTGAAG TCAACATGCC TGCCCCAAAC
2201  AAATATGCAA AAGGTTCACT AAAGCAGTAG AAATAATATG CATTGTCAGT
2251  GATGTACCAT GAAACAAAGC TGCAGGCTGT TTAAGAAAAA ATAACACACA
2301  TATAAACATC ACACACACAG ACAGACACAC ACACACACAA CAATTAACAG
2351  TCTTCAGGCA AAACGTCGAA TCAGCTATTT ACTGCCAAAG GGAAATATCA
2401  TTTATTTTTT ACATTATTAA GAAAAAAGAT TTATTTATTT AAGACAGTCC
2451  CATCAAAACT CCGTCTTTGG AAATCCGACC ACTAATTGCC AAACACCGCT
2501  TCGTGTGGCT CCACCTGGAT GTTCTGTGCC TGTAAACATA GATTCGCTTT
2551  CCATGTTGTT GGCCGGATCA CCATCTGAAG AGCAGACGGA TGGAAAAAGG
2601  ACCTGATCAT TGGGGAAGCT GGCTTTCTGG CTGCTGGAGG CTGGGGAGAA
2651  GGTGTTCATT CACTTGCATT TCTTTGCCCT GGGGGCGTGA TATTAACAGA
2701  GGGAGGGTTC CCGTGGGGGG AAGTCCATGC CTCCCTGGCC TGAAGAAGAG
2751  ACTCTTTGCA TATGACTCAC ATGATGCATA CCTGGTGGGA GGAAAAGAGT
2801  TGGGAACTTC AGATGGACCT AGTACCCACT GAGATTTCCA CGCCGAAGGA
2851  CAGCGATGGG AAAAATGCCC TTAAATCATA GGAAAGTATT TTTTTAAGCT
2901  ACCAATTGTG CCGAGAAAAG CATTTTAGCA ATTTATACAA TATCATCCAG
2951  TACCTTAAAC CCTGATTGTG TATATTCATA TATTTTGGAT ACGCACCCCC
3001  CAACTCCCAA TACTGGCTCT GTCTGAGTAA GAAACAGAAT CCTCTGGAAC
3051  TTGAGGAAGT GAACATTTCG GTGACTTCCG ATCAGGAAGG CTAGAGTTAC
3101  CCAGAGCATC AGGCCGCCAC AAGTGCCTGC TTTTAGGAGA CCGAAGTCCG
3151  CAGAACCTAC CTGTGTCCCA GCTTGGAGGC CTGGTCCTGG AACTGAGCCG
3201  GGCCCTCACT GGCCTCCTCC AGGGATGATC AACAGGGTAG TGTGGTCTCC
3251  GAATGTCTGG AAGCTGATGG ATGGAGCTCA GAATTCCACT GTCAAGAAAG
3301  AGCAGTAGAG GGGTGTGGCT GGGCCTGTCA CCCTGGGGCC CTCCAGGTAG
3351  GCCCGTTTTC ACGTGGAGCA TAGGAGCCAC GACCCTTCTT AAGACATGTA
```

FIGURE 7 (c)

3401 TCACTGTAGA GGGAAGGAAC AGAGGCCCTG GGCCTTCCTA TCAGAAGGAC

3451 ATGGTGAAGG CTGGGAACGT GAGGAGAGGC AATGGCCACG GCCCATTTTG

3501 GCTGTAGCAC ATGGCACGTT GGCTGTGTGG CCTTGGCCAC CTGTGAGTTT

3551 AAAGCAAGGC TTTAAATGAC TTTGGAGAGG GTCACAAATC CTAAAAGAAG

3601 CATTGAAGTG AGGTGTCATG GATTAATTGA CCCCTGTCTA TGGAATTACA

3651 TGTAAAACAT TATCTTGTCA CTGTAGTTTG GTTTTATTTG AAAACCTGAC

3701 AAAAAAAAAG TTCCAGGTGT GGAATATGGG GGTTATCTGT ACATCCTGGG

3751 GCATTAAAAA AAAATCAATG GTGGGGAACT ATAAAGAAGT AACAAAAGAA

3801 GTGACATCTT CAGCAAATAA ACTAGGAAAT TTTTTTTTCT TCCAGTTTAG

3851 AATCAGCCTT GAAACATTGA TGGAATAACT CTGTGGCATT ATTGCATTAT

3901 ATACCATTTA TCTGTATTAA CTTTGGAATG TACTCTGTTC AATGTTTAAT

3951 GCTGTGGTTG ATATTTCGAA AGCTGCTTTA AAAAAATACA TGCATCTCAG

4001 CGTTTTTTTG TTTTTAATTG TATTTAGTTA TGGCCTATAC ACTATTTGTG

4051 AGCAAAGGTG ATCGTTTTCT GTTTGAGATT TTTATCTCTT GATTCTTCAA

4101 AAGCATTCTG AGAAGGTGAG ATAAGCCCTG AGTCTCAGCT ACCTAAGAAA

4151 AACCTGGATG TCACTGGCCA CTGAGGAGCT TTGTTTCAAC CAAGTCATGT

4201 GCATTTCCAC GTCAACAGAA TTGTTTATTG TGACAGTTAT ATCTGTTGTC

4251 CCTTTGACCT TGTTTCTTGA AGGTTTCCTC GTCCCTGGGC AATTCCGCAT

4301 TTAATTCATG GTATTCAGGA TTACATGCAT GTTTGGTTAA ACCCATGAGA

4351 TTCATTCAGT TAAAAATCCA GATGGCGAAT GACCAGCAGA TTCAAATCTA

4401 TGGTGGTTTG ACCTTTAGAG AGTTGCTTTA CGTGGCCTGT TTCAACACAG

4451 ACCCACCCAG AGCCCTCCTG CCCTCCTTCC GCGGGGGCTT TCTCATGGCT

4501 GTCCTTCAGG GTCTTCCTGA AATGCAGTGG TCGTTACGCT CCACCAAGAA

4551 AGCAGGAAAC CTGTGGTATG AAGCCAGACC TCCCCGGCGG GCCTCAGGGA

4601 ACAGAATGAT CAGACCTTTG AATGATTCTA ATTTTTAAGC AAAATATTAT

4651 TTTATGAAAG GTTTACATTG TCAAAGTGAT GAATATGGAA TATCCAATCC

4701 TGTGCTGCTA TCCTGCCAAA ATCATTTTAA TGGAGTCAGT TTGCAGTATG

4751 CTCCACGTGG TAAGATCCTC CAAGCTGCTT TAGAAGTAAC AATGAAGAAC

4801 GTGGACGTTT TTAATATAAA GCCTGTTTTG TCTTTTGTTG TTGTTCAAAC

FIGURE 7 (d)

```
4851  GGGATTCACA GAGTATTTGA AAAATGTATA TATATTAAGA GGTCACGGGG
4901  GCTAATTGCT AGCTGGCTGC CTTTTGCTGT GGGGTTTTGT TACCTGGTTT
4951  TAATAACAGT AAATGTGCCC AGCCTCTTGG CCCCAGAACT GTACAGTATT
5001  GTGGCTGCAC TTGCTCTAAG AGTAGTTGAT GTTGCATTTT CCTTATTGTT
5051  AAAAACATGT TAGAAGCAAT GAATGTATAT AAAAGC
```

FIGURE 7 (e)

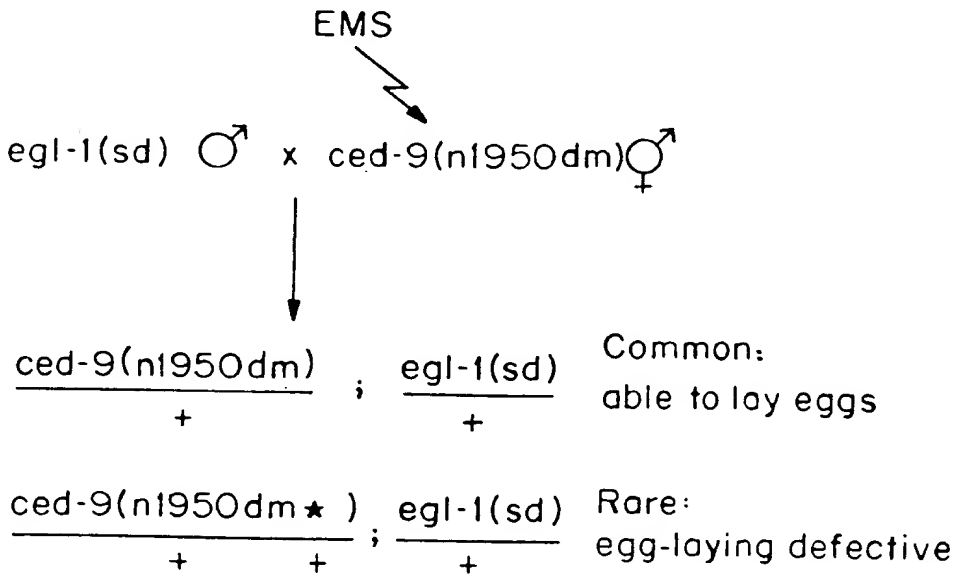
EMS
egl-1(sd) ♂ x ced-9(n1950dm)♀
ced-9(n1950dm) / + ; egl-1(sd) / +
Common:
able to lay eggs
ced-9(n1950dm★) / + + ; egl-1(sd) / +
Rare:
egg-laying defective

FIG. 8

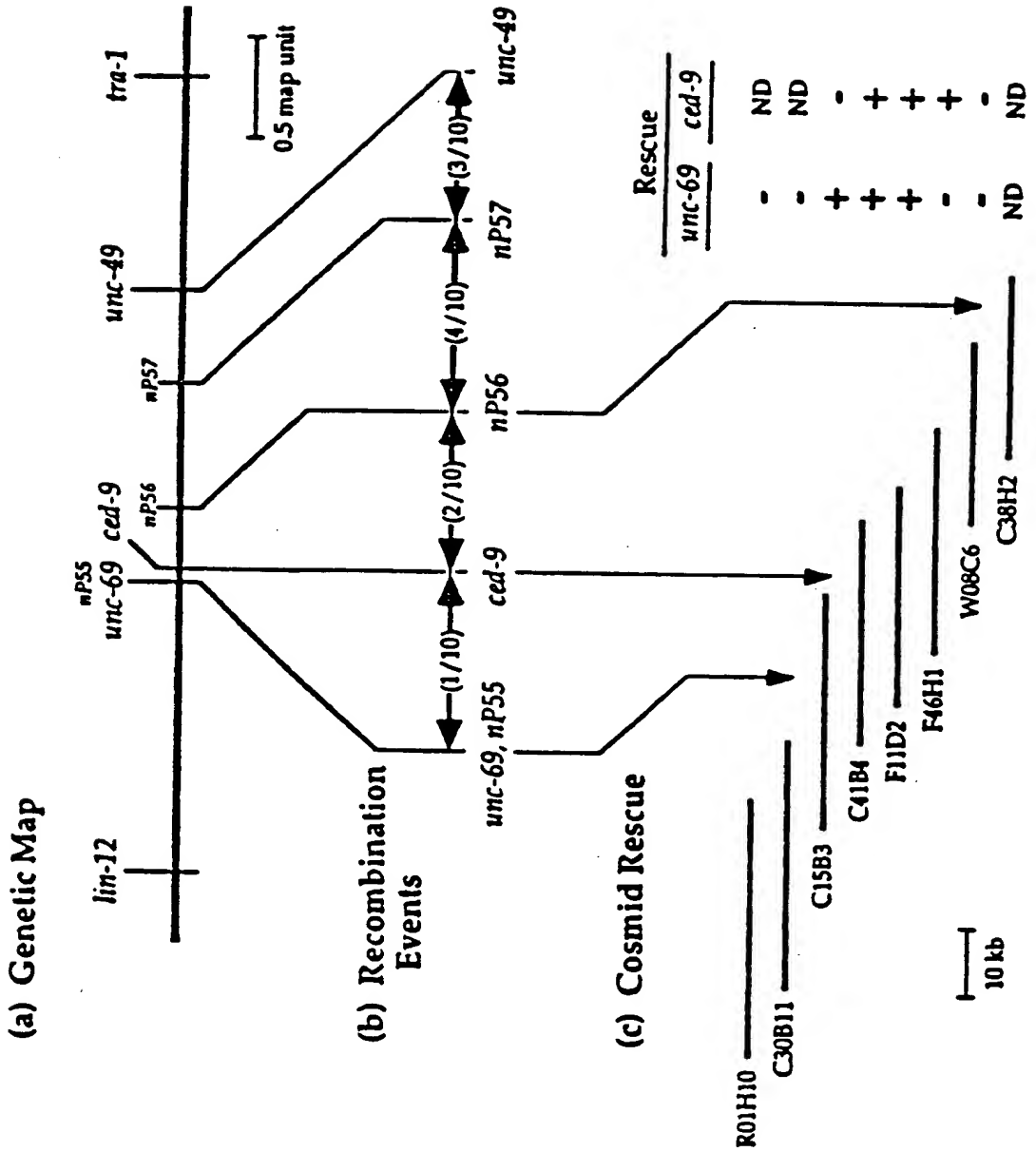
(a) Genetic Map
lin-12
unc-69
nP55
ced-9
nP56
nP57
unc-49
tra-1
0.5 map unit
(b) Recombination Events
unc-69, nP55
(1/10)
ced-9
(2/10)
nP56
(4/10)
nP57
(3/10)
unc-49
(c) Cosmid Rescue
Rescue
unc-69 ced-9
ND ND
- -
- -
+ +
+ +
+ +
- -
- -
ND ND
R01H10
C30B11
C15B3
C41B4
F11D2
F46H1
W08C6
C38H2
10 kb

FIGURE 9

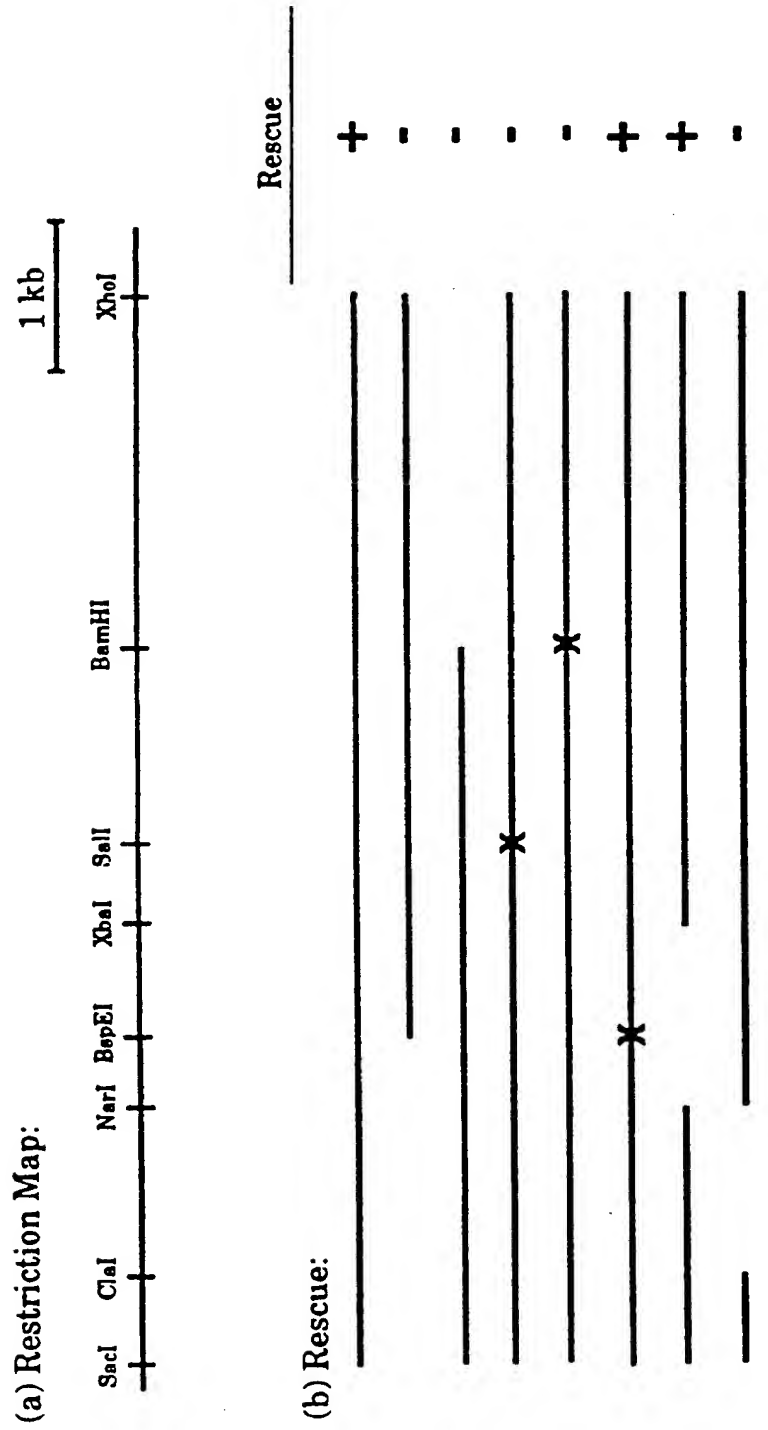
(a) Restriction Map:
1 kb
SacI
ClaI
NsrI
BspEI
XbaI
SalI
BamHI
XhoI
(b) Rescue:
Rescue
(c) Transcripts (cDNAs):
0.75 kb
2.1 kb
1.3 kb
SL1
SL1

FIGURE 10

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 93/05651

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12N15/12; C12N15/11; C12Q1/68; C07K13/00
A61K31/70; A61K37/02; G01N33/577

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C12N ; C07K ; C12Q ; A61K G01N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
Y	NATURE vol. 356, 9 April 1992, MACMILLAN JOURNALS LTD., LONDON,UK; pages 494 - 499 M.O. HENGARTNER ET AL. 'Caenorhabditis elegans gene ced-9 protects cells from programmed cell death' see page 494, left column, line 1 - page 499, right column, line 46 ---	1-12, 18-20, 22-25, 27-42, 45-114
Y	NATURE vol. 335, 8 September 1988, MACMILLAN JOURNALS LTD., LONDON, UK; pages 184 - 186 A. COULSON ET AL. 'Genome linking with yeast artificial chromosomes' see page 184, left column, line 1 - page 186, left column, line 22 --- -/--	1-12, 18-20, 22-25, 27-42, 45-114

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
04 OCTOBER 1993	14 -10- 1993
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	HORNIG H.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
Y	NATURE vol. 335, 28 September 1988, MACMILLAN JOURNALS LTD., LONDON,UK; pages 440 - 442 D.L. VAUX ET AL. 'Bcl-2 gene promotes haemopoetic cell survival and coorüerates with c-myc to immortalize pre-B cells' cited in the application see page 440, left column, line 1 - page 442, right column, line 15	13-17, 21-23, 26-40, 43-46, 48-114
Y	CELL vol. 67, no. 5, 29 November 1991, CELL PRESS, CAMBRIDGE, MA,US; pages 889 - 899 A. STRASSER ET AL. 'Bcl-2 transgene inhibits T cell death and perturbs thymic self-censorship' cited in the application see page 890, left column, paragraph 2 - page 895, left column, paragraph 1	13-17, 21-23, 26-40, 43-46, 48-114
Y	CELL vol. 67, no. 5, 29 November 1991, CELL PRESS, CAMBRIDGE, MA,US; pages 879 - 888 C.S.SENTMAN ET AL. 'Bcl-2 inhibits multiple forms of apoptosis but not negative selection in thymocytes' cited in the application see page 879, left column, line 1 - page 880, left column, line 10	13-17, 21-23, 26-40, 43-46, 48-114
Y	ANNUAL REVIEW OF CELL BIOLOGY vol. 7, 1991, ANNUAL REVIEW INC.,CALIFORNIA,US; pages 663 - 698 R.E. ELLIS ET AL. 'Mechanisms and functions of cell death' cited in the application the whole document	1-114
A	EP,A,0 252 685 (THE WISTAR CORPORATION) 13 January 1988 the whole document	-
P,Y	WO,A,9 300 909 (REGENERON PHARMACEUTICALS, INC.) 21 January 1993 see claims 1-98 see page 31, line 15 - page 41, line 27	52-61

-/--

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
P,Y	SCIENCE vol. 258, 18 December 1992, AAAS, WASHINGTON,DC,US; pages 1955 - 1957 D.L. VAUX ET AL. 'Prevention of programmed cell death in Caenorhabditis elegans by human bcl-2' see page 1955, left column, line 1 - page 1956, middle column, line 28 -----	13-17, 21-23, 26-40, 43-46, 48-114

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/05651

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark : Although claims 36-61 and 65-69 (as for as they concern in vivo treatment of human or animals) and claims 62-64 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 9305651 SA 76373

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 04/10/93
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0252685	13-01-88	US-A-	5015568	14-05-91
		AU-B-	602704	25-10-90
		AU-A-	7532887	18-02-88
		DE-A-	3786200	22-07-93
		JP-A-	63100379	02-05-88
		US-A-	5202429	13-04-93
WO-A-9300909	21-01-93	AU-A-	2322392	11-02-93